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ect of certain proanthocyanidins and catechins on the nucleic aci l nitrogen contents of *Lemna paucicostata* Hegelm.

S SEETA RAM RAO and K V N RAO*

Department of Botany, P G College of Science, Osmania University, Saifabad, Hyderaba 500 004, India

* Department of Botany, Osmania University, Hyderabad 500 007, India

MS received 7 January 1983; revised 19 October 1983

Abstract. Effect of proanthocyanidins and catechins isolated from diverse plant sources w studied on the growth of *Lemna paucicostata* Hegelm. There was no difference in DN contents of *Lemna* plants in any of the treatments. Soluble nitrogen content was less at t concentrations promoting maximum growth of *Lemna* plants as compared to the contr RNA and protein nitrogen contents increased as growth was promoted by certain concentr tions of proanthocyanidins and catechins. The possibility of growth regulation by pr anthocyanidins and catechins through nucleic acid and nitrogen metabolism is discussed

Keywords. Proanthocyanidins; catechins; nucleic acid; nitrogen; *Lemna paucicostata*.

Introduction

studies on the growth promoting activities of coconut milk, the liquid endosperr *Cocos nucifera* and the fluid extracts from the unripe fruits of horse chestnu *culus woerlitzensis* revealed that one of the growth promoting factors in thes ids associated with the developing embryos was leucoanthocyanin, now referred t roanthocyanidin (Shantz and Steward 1955; Steward and Shantz 1956, 1959) ng carrot explants, Shantz and Steward (1955) and Steward (1968) reported th vth promoting activities of leucoanthocyanins isolated from different plan rces. Earlier, the growth promoting activities of proanthocyanidins and catechin ited from diverse plant sources on the growth of an angiospermic whole plan em represented by *Lemna paucicostata* Hegelm. has been established (Rao *et a*)). In the present paper, the effect of proanthocyanidins on the nucleic acid an ogen contents of *L. paucicostata* are communicated

Lemna plants were grown on Bonner and Devirian medium witho temperature of 25±1°C and light intensity of 5000 lux. The effect of was tested at five concentrations *viz*. 0·01, 0·05, 0·1, 0·5 and 1·0 ppm. Gr fresh weight was measured at the end of 10 days (for details see Rao *et a* concentrations at which maximum and minimum induction of gro increase in fresh weight was observed, were used for the determinati soluble nitrogen and protein nitrogen. *Lemna* plants were homogenize ethyl alcohol.

2.1 *Nucleic acid estimation*

DNA and RNA fractions present in the homogenate were separate procedure described by Ogur and Rosen (1950). The quantitative es was done according to the procedure of Burton (1968). RNA in determined using the procedure of Schneider (1957).

2.2 *Nitrogen estimation*

Lemna plant homogenate was centrifuged. The supernatant was the s nitrogen and the sediment was the source of protein nitrogen. Solul estimated employing the procedure described by Moore and Stein (19 the protein content, the sediment was transferred to a corning test tub HCl was added to it. The open end of tube was sealed. Such sealed tube to 14 hr of autoclaving, to ensure complete hydrolysis of prote formation of humic acids. On cooling, the tapered ends of the tubes w the contents was filtered through Whatman No. 42 filter papers. hydrolysate was removed by gently heating on a sand bath at 70°C. T filtrate was made up to a standard volume. The nitrogen present in using the procedure by Moore and Stein (1954).

3. Results

The effects of proanthocyanidins and catechins on the nucleic ac contents at the two concentrations (concentration A, where maxim induced and concentration B, where minimum growth was induce table 1.

There was no difference in the DNA content in the treatments and the the compounds the RNA content was higher at concentration A than a

hocyanidins and catechins on the nucleic acid and nitrogen contents of *L. paucicostata*.

n *A* e owth and	Concentration *B* in ppm where minimum growth was induced and fresh weight at that concentration in mg*	DNA content in mg/g fresh weight**		RNA content in mg/g fresh weight**		Soluble nitrogen in μg/g fresh weight**		Protein nitrogen in μg/g fresh weight**	
		Conc. *A*	Conc. *B*	Conc. *A*	Conc. *B*	Conc. *A*	Conc. *B*	Conc. *A*.	Conc. *B*.
	0·01–683	0·84	0·84	12·60	7·65	45·0	65·0	962	762
	0·01–825	0·96	0·88	14·54	10·50	45·0	72·5	1037	862
	0·01–773	0·96	0·84	12·15	9·00	47·5	75·0	1000	762
	0·01–603	0·84	0·88	8·40	7·05	55·0	72·5	850	537
	0·01–676	0·84	0·88	11·85	7·35	65·0	72·5	762	500
	1·0 –522	0·96	0·88	9·00	6·30	52·5	82·5	687	387
	0·01–690	0·84	0·84	13·95	7·65	47·5	72·5	975	737
	1·0 –447	0·96	0·96	8·10	5·55	75·0	92·5	812	387
589		0·88		6·60		82·5		440	

** Each value represents the mean of 3 replicates.

4. Discussion

There are several references in the literature linking the activities of natur growth regulators with the nucleic acid metabolism (Key 1969). It proanthocyanidins and catechins, which promote the growth of *Lemna* *et al* 1980) affect the growth through nucleic acid metabolism as envisa RNA contents. There is a linear relationship between the increase in gro content. Based on the observations of Thimann and Radner (1955 anthocyanin formation in *Spirodela oligorrhiza* was linked with the nucleotides, Steward and Shantz (1959) suspected that leucoanthocyan phenolic compounds exert their regulatory activity by affecting the synth acids and proteins.

The higher RNA levels in *Lemna* plants treated with the compound increased the protein synthesis because, the concentrations where max occurred were associated with high levels of RNA and protein nitrogen. T of the stimulation of growth with the active fraction of *Aesculu* leucoanthocyanins (proanthocyanidins) and other cell division inducin was reported by Steward and Rao (1971). These authors have also obse levels of nucleic acids and protein nitrogen in the treated carrot explants. proanthocyanidins and catechins present in appropriate physiological c in the nutrient medium, might have enhanced the absorption of nitrogen further assimilation into protein, by increasing the levels of RNA in the Recently Marschner (1978) and Marschner *et al* (1974) suggested compounds produced by plant roots increased the absorption of iro plants. Thus the role of phenolics in the absorption of other elements ne investigation.

The observation of Hillis (1955) regarding abundant occurrence thocyanins in the young leaf tips and secondary cambial cells of eucalyp spring flush of growth also support the view that proanthocyanidins growth by increasing protein synthesis.

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Overlooked exomorphological evidences towards the cor clature of the so-called *Nechamandra alternifolia* (Roxb.

E GOVINDARAJALU

Department of Botany, Presidency College, Madras 600 005, India

MS received 7 April 1983

Abstract. One of the aquatic monocotyledonous taxa commonly distr India and Ceylon has been placed under *Vallisneria, Lagarosiphon* and *Ne* indicative of its unsettled nomenclature. This is now clarified and solved discovery of the staminodes that have been overlooked and/or misinterp authors. The basis for this baffling situation resulting in the nomenclatur different descriptions of this taxon as given by the earlier authors have b reference to the present observations and interpretations. The summation on the newly discovered exomorphological structures (staminodes) st interpretations as supported by and relevant to the traditional theoretical *nomen* of the taxon under debate is established as *Lagarosiphon alternifc* Emended description and economic importance are given.

Keywords. *Nechamandra alternifolia*; staminodes; *Lagarosiphon alterni* omorphological evidences.

1. Introduction

A diligent perusal of the taxonomic description and the synonyms o *alternifolia* (Roxb.) Thw. (Bas. *Vallisneria alternifolia* Roxb.) clearly there has been considerable amount of confusion right from the beginni its correct nomenclature and characterization. This confusion seems to primarily for want of correct understanding of its basic floral morph result that this taxon has undergone considerable number of nomenclat shuntings and viscissitudes from time to time (Subramanyam and Balal Thus the genus *Nechamandra* ever since it was erected by Planchon (An 3, 11: 78, 1849) as one of the genera under Hydrocharitaceae seems to

subgenus under *Vallisneria* but recognized the species as *V. altern*
synonymizing at the same time *N. roxburghii* Planch. and *L. roxburg*
Benth. under the latter. It is rather unfortunate that the correct nomenc
common species widely distributed in Indian freshwaters still remains u
and unsettled.

2. Materials and methods

The flowers of the Hydrocharitaceae are known for their deliquescen
cannot be analysed after drying. Therefore neither has any suitable nor sp
of dissection and critical examination of fresh flowers been employed l
authors, instead their observations were based perhaps on herbariu
Therefore taking the built in deficiency of the flowers into considera
circumstances under which this taxon might have been studied earlier
importance has now been given for the examination of fresh and pickled
Furthermore, to render the white and minute floral parts more clear a
better perceptibility the flowers were stained with a dilute solution of s
flowers at the stage of anthesis were also collected and studied to avoid ris
any damages to them while dissecting.

Nearly 500 male and female flowers (mostly in the anthesis stage) w
from different areas within Tamilnadu and analyzed following the
detailed above. Male flowers randomly picked from different spathes of d
plants were studied. Besides this the flowers that were released successiv
male plants grown in a tank under laboratory conditions were also ma

3. Natural history

Nechamandra alternifolia is common and thrives well in almost all fresh
ponds and semipermanent water sheds and is characterized by stolonifer
monopodial growth (figures 1, 2). Because of this particular growth habit
the vegetative propagation caused by means of fragmentation of stems
followed by their dissemination this species gains supremacy in course
other hydrophytes and becomes ultimately dominant in a given area w
period.

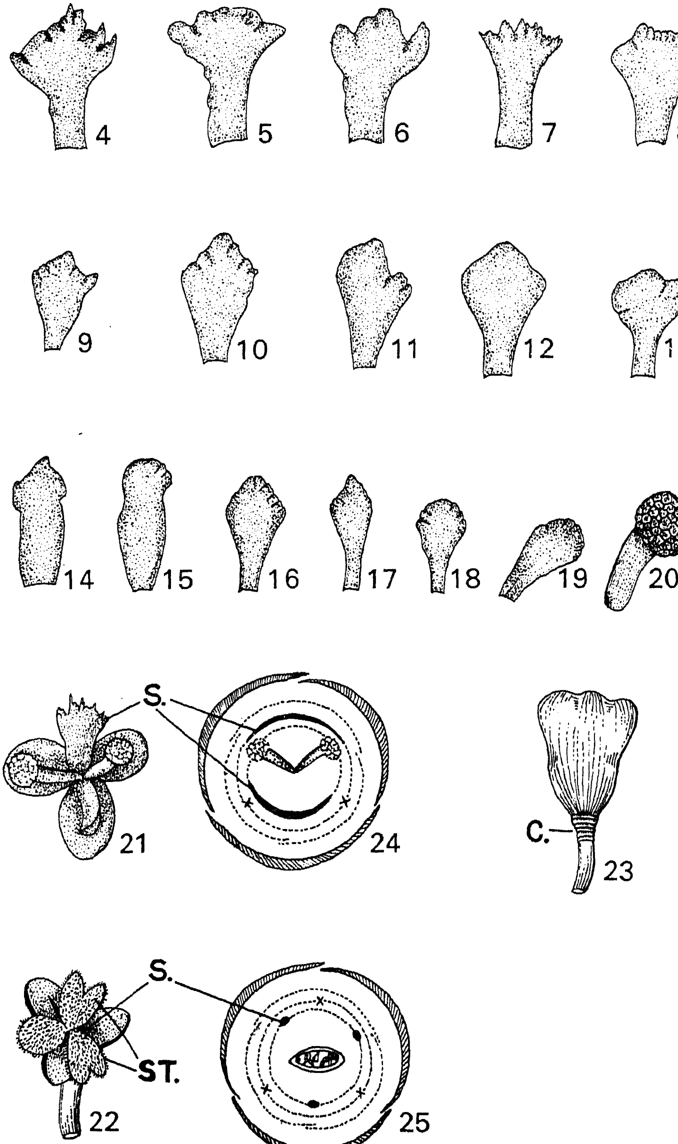
The congested pseudoverticillate arrangement of the falcate usually t

Figures 1–3. *Lagarosiphon alternifolia* (Roxb.) Druce **1,2.** Habit of female and m
plants respectively (× 1/4). **3.** Papillate stigmatic lobes and the staminodes (×
(s-staminodes; ST-stigmatic lobes).

Observations and discussion

1 *Male flowers*

he flowers are minute, colourless or pinkish at apex, obtuse, truncate or trilob
ate to obovate, pedicellate, marked by a narrow zone of few distinct annu
arkings caused by constrictions at the junction of the pedicel and the perianth (fig
) which appears to facilitate later their disarticulation and distribution. The pedic
e somewhat oblique with respect to the peduncle. Each spathe usually contains on
erage 90–105 flowers. The spathe is made up of a pair of ovate-elliptic greeni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them in a single flower nor between those of any two flowers in the same or in spathes is comparable and similar in respect of their size and shape (figure

4.2 *Interpretations and taxonomic value of inner tepal like structures*

The obvious and mere inner position of this pair of tepal like structures on the coupled with the tepal like appearance on the other might have easily misle earlier authors to consider them as representing the inner perianth. In fact, wl inner structures are more carefully examined flowerwise with respect to their in relation to the outer tepals it is certain that the earlier erroneous interp becomes obvious. One pertinent question that may be raised is that when always three tepals comprising the outer perianth in all the staminate flowers any exception and kinds of variation in their number, size and shape why is it so-called inner perianth alone should consist of just two members only (chas ous flowers) and one (cleistogamous flowers) associated with considerable var size and shape in every sample? In other words the occurrence of trimery in whorl and dimery in the inner whorl seems to conflict with and is paradoxic theoretical understanding of the perianth condition in the family as a wh existence of dimery in the inner whorl may easily be reconciled by assuming th the three members might have been lost due to the phylogenetic impact. Gran this is true the question of their anomalous position with respect to those of whorl cannot be overlooked and thus remains unsolved.

Even in regard to the members of the perianth and their number, stam staminodes there appears to be a lot of conflicting statements in all the earlie Therefore it is worthwhile to scan through all the earlier account of the male fl *Nechamandra alternifolia* as well as that of other taxa that are considere synonymous with it.

Vallisneria alternifolia Roxb. The perianth is said to possess 3 + 2 tepals (R 1832), 2 + 4 (Wight 1838) or only one whorl of 3 members (Drury 1869) (Ascherson and Gürcke 1889). No staminode has been mentioned (Roxbur Wight 1838). According to Drury (1869) the number of staminodes is said to be which 3 are unequal and one alternating with the sepal. There are 2 fertile *sensu* Roxburgh (1832); Wight (1838) and 3 stamens or 2-1 due to abortion (Dru

Lagarosiphon alternifolia (Roxb.) Druce. Haines (1924) and Fischer (19 mentioned the occurrence of 2 whorls of trimerous perianth, 2–3 staminodes fertile stamens.

Lagarosiphon roxburghii (Planch.) Benth. The perianth consists of 3 + 3

Thus it is seen from the foregoing conflicting accounts that only the inner structures besides other kinds of discrepancy in the descriptions that ha variously interpreted by different authors seem to be responsible for the baffling situation.

As stated earlier the flowers in the Hydrocharitaceae are cyclic (Lawrence 19 in the light of this fact the inner pair of so-called tepaloid structures despi superficial resemblance and variations when compared with those of the oute are always disposed in antero-posterior plane or in other words they diametrically opposite to each other (figures 21, 24). This kind of disposition re such a condition that one of them occupies a position opposite to and subte the larger anterior tepal of the outer whorl while its counterpart in the poster alternates with and is situated between the smaller lateral tepals of the same thereby, the alignment of the so-called inner pair of tepals which is supp represent inner whorl of perianth (*sensu auct.*) is such that not only this p appears to be quite unusual and unexpected in any cyclic flowers but this si seems to be totally alien to this family on that score. Granting that these two inn are to be regarded as the only surviving pair of tepals of an otherwise trimero perianth the existing anomalous positional relationship with respect to those outer whorl on the one hand and their consistent opposite alignment with re each other on the other presents stumbling block in considering them as equiv the two tepals of the inner perianth. In other words the inner anterior tepaloid s despite the suppression of the third member should alternate with the outer te can never remain exactly opposite to the anterior outer tepal (figures 21, 24

Secondly it is seen that the size and shape of the two inner members i stabilized but exhibits a wide spectrum of variation which is as much interesti seems to be significant. No one pair from any given flower resembles that of flower either in the same or in different spathes in regard to its shape and siz there any similarity between the two inner tepal like structures within the same Now in the light of this observation should they be still considered as represen inner perianth? It is not only difficult to believe but to account for the existence a wide qualitative and quantitative variations resulting in bizarre forms and t being always restricted only to the so-called inner perianth (figures 4–19) importance of which may be appreciated when this peculiar situation of the compared with that of the outer one which is characterized by consistent config and size (compare figures 4–19 with 21).

Thirdly the tepaloid structures of the inner whorl exhibiting flowerwise mo

unusual position. However all of them irrespective of the degree of modific
size and shape gradually attenuate towards their bases in the lower half thus
in a stalk like portion which resembles the filament part of the fertile stamen (fi
Likewise the distal half becomes demarcated by constriction and gradually r
assuming ultimately spherical to subspherical shape and rough or convolute
but devoid of pollen (figure 20). Therefore in the light of the foregoing e
namely high degree of variability in the inner pair of structures resulting ulti
stamen like structures coupled with their anomalous positional relations
respect to the outer perianth members and their reduction to 2 members wit
flower it is but logical to consider them as nothing but a pair of sta
masquerading as tepaloid structures. Under this circumstance it follows that
flower there can be only one whorl of perianth consisting of three free tepals
two whorls as has hitherto been supposed and described by the earlier auth
supra). Having thus gained an understanding of the correct morphological r
the inner pair of structures in male flowers as equivalent to a pair of stamin
next problem that is to be solved is about their anomalous position
phenomenon of oligomery in them. The theoretical assumption is that the mal
conform to the formula $P_{3+3}\,A_{3+3}\,G_0$. As seen from the floral diagram (figur
inner whorl of perianth which should have been present in this taxon in co
with the general floral theme of construction is totally absent and its th
location with respect to the outer is indicated by dotted line in figure 24. Bein
flower should the members of the inner whorl be present as perianth then the
only alternating with those of the outer. Likewise out of six stamens whic
occur in two trimerous whorls alternating with each other (outer and in
posterior stamen of the outer whorl is supposed to be modified into one st
which becomes alterniphyllous in as much as it is alternating with the latera
outer tepals while the anterior stamen of the inner whorl into another stamino
is a structure subtended by the anterior outer tepal thus becoming antephy
shown in figure 24. The two lateral stamens of the outer whorl are suppressed
all the three stamens of the inner whorl are represented in terms of one
staminode plus two posterior fertile stamens the latter two of course converge
centre to occur in pairs and united at base (figure 21). This interpretation
theoretical theme of construction and organization of the androecium see
perfectly in tune with and accounts for the normal arrangement of two wh
trimerous androecium in a cyclic flower (figure 24). Furthermore it is seen
inner anterior tepal like structure (staminode) is being embraced by tha

4.3 *Female flowers*

As in male flowers the outer perianth in the female flowers is also made up of 3 subequal tepals thus rendering them slightly zygomorphic. Following this whorl no other tepaloid structures similar to those of the inner whorl of the male flowers are present here. On the contrary very minute relatively slender two or three linear (filamentous) (figure 3) or club-shaped structures are present opposite to the tepals (antephyllous) but at the same time alternating with the three prominent stigmatic lobes (figures 22, 25). These structures are juxtaposed to and frequently adpressed with the papillate stigmatic lobes. Because of their minute size (almost microscopic) and their adherence with the stigmatic lobes and their being camouflaged on that score by the latter they might have been overlooked by the earlier authors.

In all the earlier accounts only one whorl of perianth has been ascribed. Although Hooker (1888) who has considered *N. alternifolia* (Roxb.) Thw. and *N. roxburghii* Planch. under *L. roxburghii* (Planch.) Benth. has mentioned in the key that the genus *Lagarosiphon* in contrast to *Hydrilla* possesses a single whorl but as far as the general description is concerned he has reported both for the male and the female flowers two whorls of perianth differentiated into calyx with petaloid sepals and corolla with shorter petals. According to Harvey (1842) the author of the genus *Lagarosiphon* there are two distinct whorls of trimerous perianth in flowers of both sexes. Thus the characterization of *Lagarosiphon* seems to be at variance with each other.

The two whorls of perianth for male flowers and only one whorl for female flowers as described by Subramanyam and Balakrishnan (1961), Subramanyam (1962) and several others do not satisfy theoretical understanding because when the male flowers are credited with two whorls of perianth (accepting their own statements) why is it that the female flowers alone should possess only one whorl of perianth? In short, two whorled perianth for flowers of one sex and one whorled perianth for those of another sex of the same species seems to be rather untenable.

4.4 *Status of the filament like structures*

As mentioned above the position of the filament like structures with reference to the tepals and to the three papillate stigmatic lobes is such that they always stand opposite to the former i.e. antephyllous instead of being alterniphyllous but alternating with the latter (figures 22, 25). Furthermore the number of such structures in different female flowers seems to vary from 2 to 3. On this basis it is but logical to consider these structures as representing the staminodes belonging to the inner whorl (outer whorl being completely suppressed) and not the inner perianth which is negated further by the anomalous positional relationship with respect to the whorl of outer perianth and that of the carpels. The same logic and litany advocated and advanced earlier for clarifying the morphological nature of inner tepal like structures of the male flowers holds good in respect of the female flowers also with the result it becomes possible to conclude that there is only a single whorl of perianth and 2 to 3 antephyllous staminodes belonging to the third inner whorl (figures 3, 22, 25). As far as the presence of staminodes in the male and female flowers is concerned it may be pointed out that in the former the two staminodes belong to the outer and inner whorls of stamens respectively while in the latter they all represent the inner whorl only since the outer whorl is totally suppressed.

4.5 *Geographical distribution*

Besides using morphological characters for distinguishing *Nechamandra* from *Lagarosiphon* Hutchinson (1959) has also made use of their respective geographical areas of distribution. According to him *Nechamandra* is said to be a native of South East Asia and North East Tropical Africa while *Legarosiphon* of tropical and South Africa. The dependence of geographical distribution for delimiting these two taxa raises one important question viz. when the monotypic genus *Nechamandra* Planch. whose basionym (*Vallisneria alternifolia* Roxb.) is known for its distribution in Indian fresh waters the mere assignment by making a new combination for the latter under a new generic cloak (*Nechamandra*) cannot render it to have an altogether different area of distribution which procedure is analogous to putting the cart before the horse! Furthermore tropical Africa appears to be a centre common to both the genera and all the earlier authors have indicated India and Ceylon for them. Granting that South East Asia is the centre of distribution for *Nechamandra* in contrast to that of *Lagarosiphon* (Hutchinson 1959) the former taxon cannot be expected in Peninsular India because Fischer (1969) has made it clear that South East Asia means a "collective name for the series of peninsulas and islands which lie to the east of India and Pakistan and to the south of China" and therefore even according to this modern concept geographical delimitation the South East Asia includes Burma, Thailand, Cambodia, Malaysia (Sumatra, Java, Borneo, Philippines, Celebes, Moluccas, Timor, W. New Guinea Irian), a small part of N. Australia. According to this recent view (Fischer 1969) the taxon *Nechamandra* appears to be an alien to Indian waters.

In conclusion it may be pointed out that the present study establishes beyond doubt that both male and female flowers of the so-called *N. alternifolia* (Roxb.) Thw. which is supposed to be the species distributed in India and Ceylon possess only one whorl of perianth, two staminodes in the former and two to three in the latter. In other words this taxon which has hitherto been characterized as one having no staminodes both in male and female flowers and two whorls of perianth (3 + 2) in the male and one whorl of three tepals in the female flowers is now demonstrated to be governed by the circumscription of the genus *Lagarosiphon* Harv. in as much as they possess staminodes and never without them. Furthermore the data on geographical distribution of these two taxa is also not helpful and cannot be relied upon. Therefore the correct name for this taxon is *Lagarosiphon alternifolia* (Roxb.) Druce whose description incorporating all the hitherto overlooked and/or misunderstood structures is presented below.

4.6 *Description of the species*

Lagarosiphon alternifolia (Roxb.) Druce—figures 1–25.

L. alternifolia (Roxb.) Druce in Rep. Bot. Exch. Cl. Brit. Is. 1916: 630, 1917; Haines, Bot. Bih. Or. 852, 1922; Fischer in Gamble, Fl. Pres. Madr. 1396, 1928; Santapau in Rec. Bot. Surv. Ind. ed. 2, 16(1): 233, 1958. *Vallisneria alternifolia* Roxb. Hort. Beng. 71, 1814 *nom. nud.* et Pl. Corom. 2: t. 165, 1802 et Fl. Ind. 3: 750, 1832; Wight, Ill. Ind. Bot. in Hook. Bot. Misc. 2: t. 11, 1838; Drury, Handb. Ind. Fl. III: 461, 1869; Ascherson and Gürcke in Die nat. Pfl. 251, 1889.

Nechamandra roxburghii Planch. in Ann. Sc. Nat. ser. 3, 11: 78, 1849; Walpers in Ann. bot. syst. III: 508, 1852; Dalz. and Gibs. Bomb. Fl. 277, 1861.

N. alternifolia (Roxb.) Thw. En. Plant. Zeyl. 332, 1864; Subramanyam and Balakrishnan, Bull. Bot. Surv. Ind. 3(1): 23, 1961; Subramanyam, Aq. Ang. 56, fig. 38, 1962.

Lagarosiphon roxburghii (Planch.) Benth. in Benth. and Hook. *f.* Gen. Pl. 3: 451, 1883; Hook. *f.* Fl. Br. Ind. 5: 659, 1888; Trim. Fl. Ceyl. 5: 124, 1893; Prain, Beng. Pl. 747, 1903; Cooke, Fl. Pres. Bomb. 2: 669, 1907; Duthie, Fl. U. Gang. Pl. 3: 174, 1920; Mitra, Fl. Pl. East. Ind. I: 4, 1958.

Herbs aquatic, submerged with upper leaves and branches floating, glabrous, dioecious, stoloniferous; stolons smooth. *Roots* fibrous, reddish. *Stems* terete, 40–100 cm long. *Leaves*, simple sessile, often twisted, amplexicaul, linear-lanceolate, translucent, serrulate, pinkish dotted, alternate throughout, sometimes the lowermost opposite, the uppermost pseudoverticillate and crowded, 3–5 nerved with transverse connections; sheathing base narrow, 3–5 mm long, open on one side. Male and female inflorescence, axillary; male inflorescence in 1–3 groups; female usually solitary, rarely in pairs. *Male spadix* ovate-elliptic, biconvex, slightly asymmetrical, subacute, subsessile, slightly winged and undulate, twisted or recurved at apex, becoming bivalved at anthesis; peduncle 0·5–0·75 mm long. *Spathaceous bracts* 2, ovate-elliptic, 9–10 mm long, 3–4 mm broad at the widest region, longitudinally and faintly striated. *Male flowers* minute, 90–105 in each spadix, densely packed up to 2/3 of spadix with upper 1/3 empty; flower buds usually obovate (ovate), 1–1·5 × 1–1·3 mm, widening towards apex, white or pinkish dotted or striped, shortly pedicellate; pedicel 0·75–1 mm long, capillary, distal end dilated with a few annular constrictions. *Perianth* in one whorl of 3 tepals; tepals undifferentiated, polyphyllous, transparent, imbricate, unequal, 1 – 1·25 × 0·5 – 0·75 mm; lateral pair of tepals small, odd anterior one slightly larger. *Staminodes* 2, petaloid, (one posterior of the outer whorl, another anterior of the inner whorl) antero-posterior, membranous, polymorphic (tepaloid to stamen like), 1 –1·25 × 0·25–0·75 mm. *Stamens* 2, central, club-shaped; filaments divergent, 0·75–1 mm long; anthers usually spherical, *c.* 0·25 mm long and broad, basifixed with granulate surface, monothecous, more or less transversely dehiscing; pollen grains few, large, usually spherical, *c.* 90 μm broad. *Female flowers* sessile, 4·5–6·0 mm long; spathaceous bracts 2, persistent, 8–9 mm long, 1–1·5 mm broad at the widest part, ovate-lanceolate, acute with recurved apex, coherent upto 2/3 its length, cupule-like. *Perianth* epigynous, 1·25–1·75 × 1·25–1·5 mm, rest as in male flowers. *Staminodes* 2–3, usually filiform, minute, antephyllous, alternating with stigmatic lobes, 0·2–0·5 mm long. *Ovary* inferior, rostrate, ovate-lanceolate, biconvex, pinkish dotted, slightly falcate, unilocular with uneven margin, 5–7 (excl. rostrum) × 1 mm broad at widest part; rostrum attenuated, almost terete, fistular, persistent, 3·3–3·8 mm long; ovules many, perspicaceous, orthotropous (curved), stalked, truncate, scattered and oriented at different angles; placentation lamellate; stigmas 3, sessile, cuneate, each bilobed, densely papillose, strongly reflexed, 2–2·5 mm long (uncoiled condition) × 2–2·5 mm at widest part. *Fruit* indehiscent, broadly ovate, winged, with persistent rostrum, 5–5·5 (excl. rostrum) × 3·5–4 mm; seeds 10–15 per fruit, black when dry, visible from without, pinkish dotted when wet, narrowly obovate, shortly mucronate, almost rounded, exendospermous, 1–1·25 × 0·5 mm; testa faintly vertically striated.

Note:—According to Wight (Ill. Ind. Bot. in Hook. Bot. Misc. 2: t. 11, 1838) it is called Jangi (Hindi), Naidulpanee (Tamil). Dr. Hamilton as mentioned by Wight (*l.c.*) says that this species (*Vallisneria alternifolia* Roxb.) is used for the purpose of refining sugar.

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Regulatory factors for *in vitro* multiplication of sandalwood tree *Santalum album* Linn.). I. Shoot bud regeneration and somatic embryogenesis in hypocotyl cultures

V A BAPAT and P S RAO*

Plant Morphogenesis and Tissue Culture Section, Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India

* Present address: Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Fed. Rep. of Germany

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Abstract. Large number of plantlets were obtained in hypocotyl cultures of *Santalum album* either through direct differentiation of shoot buds and their subsequent induction into rooted plants or through callus induction and somatic embryogenesis. Factors which triggered both these morphogenetic processes were investigated.

Keywords. Sandalwood; somatic embryogenesis; shoot bud regeneration; hypocotyl cultures, *Santalum album*

1. Introduction

Multiplication of trees has assumed great significance in recent years in view of the ever ncreasing demand for wood and wood products. Trees of improved quality combining lisease resistance are therefore required in large numbers in all programs concerned vith tree improvement and breeding. The methods in vogue have limited scope. Clonal election and multiplication using tissue and organ culture techniques offers new possibilities. Bonga (1977), Durzan and Campbell (1974), Winton (1978) and Sommer ind Brown (1979) have adequately discussed the applications of tissue and organ culture in forestry.

Santalum album (sandalwood) is of great economic value and is the source of fragrant vood and oil. The trees are conventionally propagated by seeds. A major threat to andalwood trees is the spike disease which has a devastating effect and very often ompletely eliminates the plantation. Efforts to control and eliminate the disease have een unsuccessful. It is, therefore, imperative to develop alternative techniques for apid and large scale multiplication of the species. Investigations were undertaken on andalwood with the main objective of developing techniques for clonal multiplication of the elite species which are also disease free as such trees are known to exist in andalwood plantations. This communication and the succeeding ones deal with some of the results obtained in this direction.

. Material and methods

he seed lot (obtained from Forest Research Laboratory Campus, Bangalore and Karnataka University Campus Dharwar) consisted of seeds collected from different

trees of the same region. Following sterilization in $HgCl_2$ for 5 min, the seeds were washed six times in autoclaved water and were sown on basal medium. Hypocotyl segments (5 mm) from *in vitro* grown 4 week old seedlings were excised and cultured aseptically on a basal nutrient medium whose composition (mg/l) was as follows: KNO_3 (1900), NH_4NO_3 (1650), $CaCl_2.2H_2O$ (440), $MgSO_4.7H_2O$ (370), KH_2PO_4 (170), $MnSO_4.4H_2O$ (25), H_3BO_3 (10), $ZnSO_4.7H_2O$ (13·9), Na_2EDTA (18·6), myo-inositol (100), nicotinic acid (5), folic acid (5), glycine (2), pyridoxine-HCl (0·5), thiamine HCl (0·5), biotin (0·05) sucrose (20 g/l), and agar (6 g/l). The basal medium (BM) was supplemented with auxins, cytokinins and other growth adjuvants as and when required either singly or in combinations. The pH of the medium was adjusted to 5·8 before jellying the medium with agar. The cultures were grown under continuous fluorescent light (1000 Lux) at $25 \pm 2°C$ and an rh of 55–60%.

3. Observations

Differentiation of shoot buds was readily achieved in hypocotyl cultures on BM supplemented with various concentrations of auxins and cytokinins, generally within 4 weeks of culture. Bud differentiation tended to be qualitatively and quantitatively superior on BM + Napthaleneacetic acid (NAA) (1 mg/l) as compared to Indoleacetic acid (IAA), Indolebutyric acid (IBA) and Napthoxyacetic acid (NOA) at the same concentration. With respect to cytokinins 6-benzylaminopurine (BAP) at 1 or 2 mg/l induced the optimum number of buds.

A detailed study was undertaken on the phenomenon of shoot bud differentiation in hypocotyl explants and the observations are given below. Throughout these experiments the medium used was BM + BAP (1 mg/l) unless otherwise stated.

3.1 *Bud differentiation from different regions of hypocotyl*

The entire length of the hypocotyl was divided into three equal parts designated according to its position (apical, middle and basal) and the morphogenetic responses in the explants obtained from each region was compared under identical cultural conditions. Approximately 90% of the hypocotyl explants excised from the basal portion exhibited the potential for producing buds. This potential decreased to 80 and 60% for those obtained from middle and apical regions respectively.

3.2 *Effect of age of seedling on bud formation*

Hypocotyl segments were excised from seedlings (2, 4, 6 and 8 weeks old) and cultured to observe whether age of seedling had any influence on bud formation. Data revealed that there was not much variation in bud regenerating capacity of explants excised from seedlings of different ages.

3.3 *Responses of hypocotyl to bud differentiation in liquid medium*

Liquid medium (of the same composition as that used for agar cultures) was used and hypocotyls were cultured atop a filter paper wick. Bud formation was noticeably rapid (10–15 days) as compared to agar medium where it was 20–30 days.

3.4 *Effect of position of the explant on bud differentiation*

Hypocotyl explants were kept in different positions on the nutrient medium to investigate whether polarity had any effect on bud formation. Bud formation was 100% when only root end of the explant was either dipped in the medium or even kept touching the surface of the medium. In an opposite position, i.e. shoot end in the medium bud formation dropped to about 10%. When the entire explant was embedded inside the agar medium, they turned brown, if kept in liquid medium about 20% of the explants regenerated buds (figure 1A).

3.5 *Responses of sliced hypocotyl segments*

Hypocotyl segments were split longitudinally and in one treatment the cut surface was kept in contact with the medium while in another it was kept away from the medium. Bud formation was 60% in the former position compared to 40% in the latter. In another treatment thin discs were excised from the hypocotyl and were cultured. In 70% cultures shoot buds originated from all sides of the disc (figure 1B). When discs were immersed in the liquid medium and kept stationary the explants turned brown. However, if placed in agitated liquid medium regeneration of buds was observed, but was poor.

3.6 *Rooting of shoot buds*

Rooting of *in vitro* obtained shoot buds was achieved by placing the excised shoot buds on a variety of auxin media. The plantlets grew to a height of 20 cm on all auxin combinations on agar as well as on liquid medium on filter paper bridges (figure 1 C–D). All the rooted plants were transferred to paper cups with vermiculite and grown further for three weeks before transfer to soil.

3.7 *Somatic embryogenesis in hypocotyl explants*

When the hypocotyl explants in which the regenerated shoot buds had been removed were transferred to a fresh medium of the same composition, new shoot buds arose and this process continued. However, in about 20% cultures extensive proliferation took place resulting in a large callus mass in which 4 weeks later numerous embryos of preglobular stages differentiated (figure 2A). To maintain a continuous supply of embryos as well as to enhance the embryogenic potential of somatic callus, portions of embryogenic callus were grown on BM enriched with various plant growth regulators at different concentrations. In one experiment embryogenic calli was grown in liquid medium.

IAA at 0·5 and 1 mg/l distinctly stimulated somatic embryogenesis. Higher concentrations were inhibitory for embryo formation but did not affect callus growth (figure 3A). Yeast extract (YE) (0·4%), casein hydrolysate (CH) (400 mg/l) and casamino acid (400 mg/l) were conducive to embryo differentiation. Peptone (400 mg/l) however, preferentially stimulated callus growth (table 1).

BAP enhanced callus growth but the embryogenic potential tended to be average (figure 3B). An interesting response of BAP was that in about 50% cultures shoots differentiated.

Gibberellic acid (GA) (1 mg/l) individually or in combination with auxins did not have much effect on callus growth and embryo differentiation. Among the various levels of

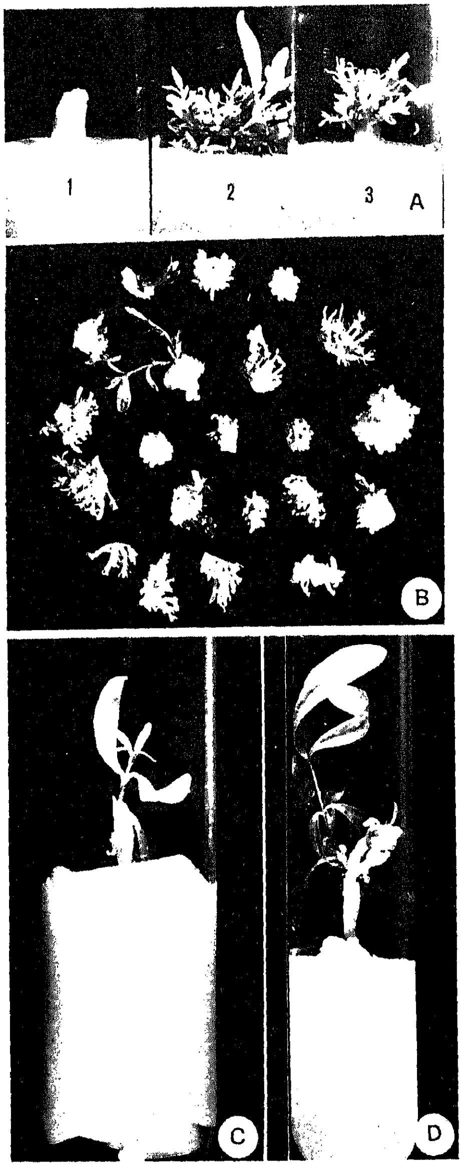

Figure 1(A–D). **A.** Effect of the position of hypocotyl on shoot bud formation **1.** Shoot end of the explant dipped in the medium. **2.** Half portion of the explant with root end in the medium. **3.** Root end of the explant just touching the medium. **B.** Slices excised from hypocotyl explant showing shoot bud regeneration. Note the degree of variation. **C.** Plantlet growing on a filter paper bridge (BM + BAP 1 mg/l). **D.** Rooted plantlet obtained from isolated shoot bud on BM + NAA (0·5 mg/l) + IBA (5 mg/l).

NH_4NO_3 a high intensity of embryogenesis was observed at 1650 mg/l of NH_4NO_3 (table 2). 2,4 dichlorophenoxy acetic acid (2, 4-D) (1 mg/l) only increased callus growth and embryo differentiation was retarded.

The effect of different carbon sources on embryogenesis was also investigated and

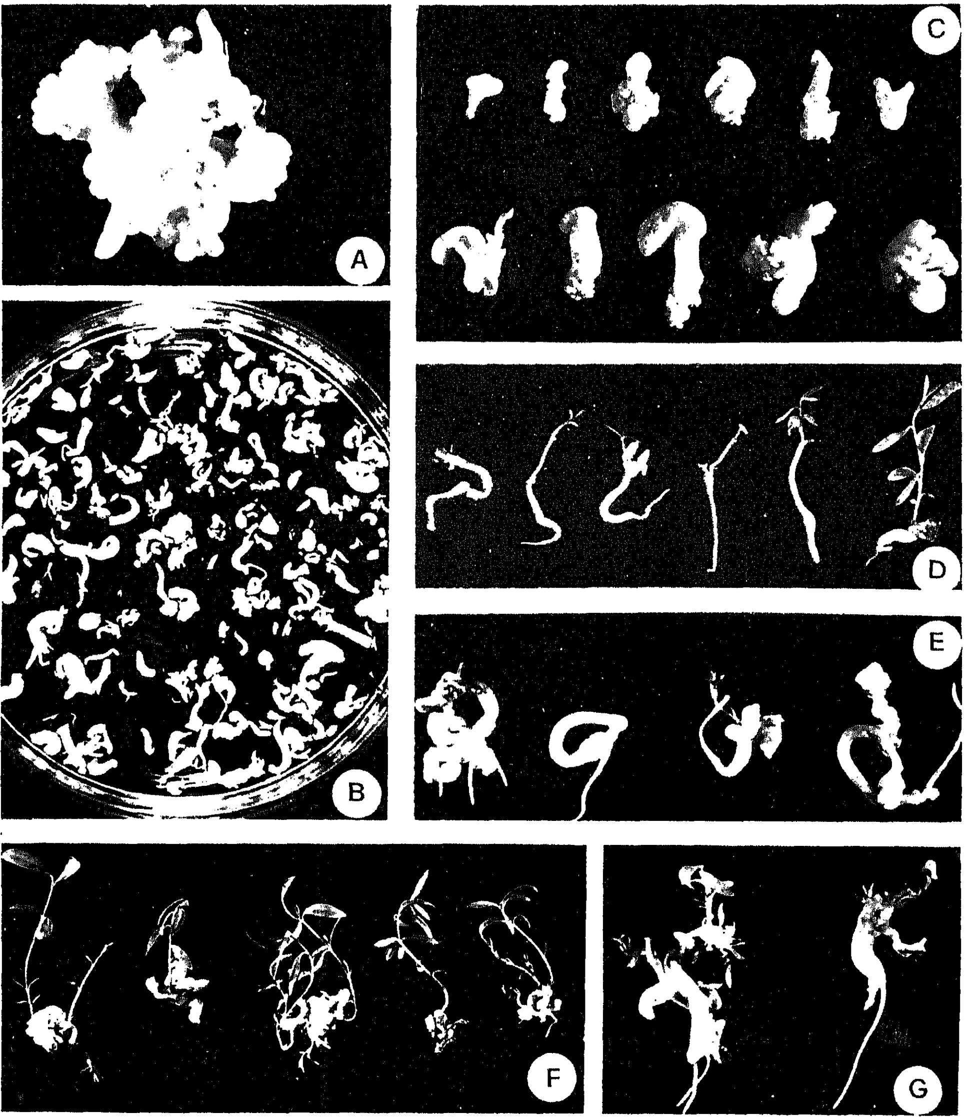

Figure 2(A–G). **A.** 4 week old callus mass on BM + BAP (1 mg/l). **B.** Isolated embryos from callus. **C.** Abnormal embryos. **D.** Normal plantlets with root and shoot development. **E.** Development of only roots from embryos and poor shoot development. **F.** Profuse shoot development and inhibition of rooting. **G.** Fasciated plantlets.

sucrose was found to be superior. A correlation was also observed between sucrose concentration and embryo differentiation (table 3). At higher levels of sucrose, roots differentiated from the callus.

Differentiation of embryos was augmented if the embryogenic callus was grown in liquid medium and agitated. In many cultures young embryos redifferentiated again and produced a callus mass which again showed prolific differentiation of embryos. A noteworthy feature was that embryo formation was a continuous, non-synchronous

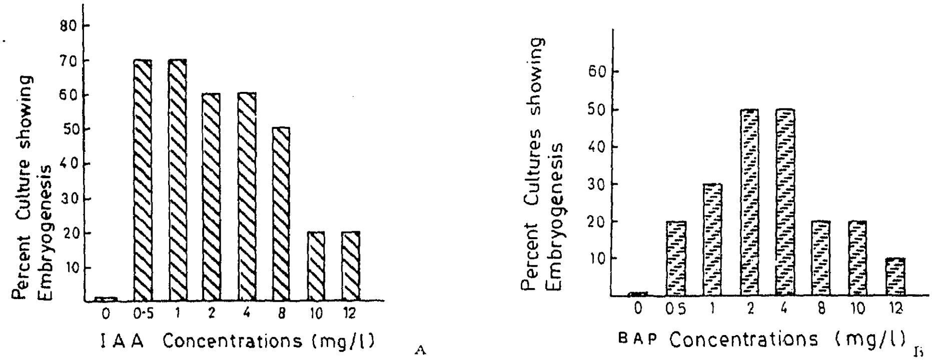

Figure 3(A–B). A. Effect of different concentrations of IAA on *in vitro* embryogenesis in *Santalum* (growth period 4 weeks). **B.** Effect of different concentrations of BAP on *in vitro* embryogenesis in *Santalum* (growth period 4 weeks).

Table 1. Effect of different combinations of IAA with various nitrogen sources on callus development and embryo differentiation in *Santalum album**.

Medium	Intensity of callus development	Intensity of embryo differentiation	% cultures producing embryo
BM + IAA	+ + +	+ + +	60
BM + IAA + YE	+ +	+ + + +	70
BM + IAA + Casamino Acid	+ + +	+ + +	70
BM + IAA + Peptone	+ + +	+ +	50
BM + IAA + CH	+ + +	+ + + +	80

* Data scored at the end of 4 weeks, 12 replicates per treatment. + + moderate, + + + high, + + + + intense. Concentrations of auxin 1 mg/l; nitrogen sources 400 mg/l.

Table 2. Effect of different concentrations of NH_4NO_3 on callus development and embryo differentiation in *Santalum album.*

Conc. of NH_4NO_3 (mg/l)	Intensity of callus development	Intensity of embryo differentiation	% cultures producing embryos
0	—	—	—
1650	+ + +	+ + +	70
3300	+ +	+ +	60
4950	+	+	30
6600	+	–	–

* Data scored at the end of 4 weeks, 10 replicates per treatment.
– nil, + low, + + moderate, + + + high.

Table 3. Effect of different concentrations of sucrose on callus development and embryo differentiation in *Santalum album**.

Sucrose conc. (%)	Intensity of callus development	Intensity of embryo differentiation	% cultures producing embryos
0	–	–	–
1	++	++	10
2	+++	+++	70
4	++++	++++	80
8	++	++	40
12	++	+	20
16	++	–	–
20	+	–	–

* Data scored at the end of 4 weeks, 12 replicates per treatment.
– nil, + low, ++ moderate, +++ high, ++++ intense.

process. The somatic callus was an assorted mixture of embryogenic masses of few cells, mature embryos and juvenile plantlets (figure 2B). Many new embryos arose by budding from other embryos. Embryogenic callus was maintained by periodic subculturing at an interval of 4–6 weeks. Over several passages of subculture there was no evidence for any decline in embryogenic potential.

Blended with normal types of embryos various abnormal embryos were also observed (figure 2c). The size and shape of these embryos varied greatly and it was not possible to observe any regularity. Several embryos of different shapes appeared in the same culture and gigantic forms were found together with dwarfs. Anomalies of embryos appeared in cultures regardless of growth substances in the culture medium and appeared to be an inherent tendency of differentiating callus. The embryogenic callus multiplied its fresh weight five to ten fold during the successive 30 days.

In order to obtain plantlets fully developed embryos were isolated and placed on BM supplemented with NAA or IAA (1 mg/l). Four types of plantlets were obtained: (i) normal plantlets with well developed root and shoot growth (figure 2D); (ii) only root growth was prominent with very poor shoot development (figure 2E); (iii) shoot development was excellent but arrested root growth (figure 2F); (iv) abnormal development of both shoot and root (figure 2G). Normal plantlets were carefully isolated and were transferred to plastic vials with vermiculite. They were irrigated with Hoagland's nutrient solution and covered with a bell jar to maintain humid condition. After 3 weeks of acclimatization under these conditions, the plantlets were transferred to soil. No host was provided as the objective was to assess whether the plants could grow without the host. Only 10 % of the plants survived under field conditions. Further efforts are underway to improve the frequency of survival of transplanted plants.

4. Discussion

Several interesting features have emerged from this study. Although bud formation was manifest in all hypocotyl cultures variations were noticed in the responses among different seedlings and more interesting were the differences observed in bud formation

in the different regions of the hypocotyl. For instance, the basal portion of the hypocotyl was more regenerative. Age of the hypocotyl had little effect on bud formation in *Santalum*. Another interesting aspect was the ability of hypocotyl explants to develop shoot buds early in liquid medium in 10–12 days compared to 30 days on the agar medium. This may be due to easy and rapid absorption of nutrients by the cultured explants compared to agar medium.

The effect of position of hypocotyl explant on the medium influenced bud formation. Maximum induction of shoot buds occurred when the explant was placed in a position in which the root end of the explant was embedded in the medium. This indicates that polarity was an important factor in determining bud formation. Butenko (1968) suggested that tissue polarity which determines the intensity of callus formation and the nature of organogenesis is the result of polar transport of growth substances, primarily auxins and possibly the result of a gradient of enzyme activity.

The size of the explant did not determine the extent of shoot bud formation in *Santalum* as evidenced by bud formation even in thin discs.

As interesting feature of the present study was the potential of the hypocotyl explant to embark upon embryogenesis *via* callus formation. It was all the more intriguing that somatic embryogenesis occurred in explants in which shoot buds had been removed and were transferred to a fresh medium. This shift in the morphogenetic expression from an organogenetic phase occurring in the same explant under the same nutritional and environmental conditions, is to our knowledge the only report although diverse morphogenetic responses are obtained in the same explants by varying growth regulators added to the medium as in *Petunia* (Rao *et al* 1973), *Antirrhinum* (Rao and Harada 1974) *Citrus* (Chaturvedi and Mitra 1975) and *Atropa* (Konar *et al* 1972).

Among the several plant growth regulators, IAA remarkably stimulated embryogenesis, followed by BAP. Other hormones were not effective. In an earlier investigation, Rao and Rangaswamy (1970) described embryogenesis in embryonal cultures of *Santalum album* on a medium fortified with yeast extract, kinetin and 2,4-D. Lakshmi Sita *et al* (1979) reported embryoid differentiation in shoot callus cultures of *S. album* on a medium supplemented with GA.

Sucrose at 4% was found to be very effective for embryogenesis, but higher levels brought a decline in the embryogenic potential of the callus. Ammirato and Steward (1971) have reported that higher levels of sucrose (120 g/l) retarded the growth of embryos whereas lower concentrations allowed the embryos to develop more leafy cotyledons. It therefore appears that osmotic concentration of the nutrient medium is one of the important factors regulating embryogenesis.

Alongside normal embryogenesis the presence of abnormal embryos was also observed in the present study. Abnormal embryoids have been reported in tissue cultures of carrot and their occurrence has been attributed to the hormonal conditions of the medium (Nakajima and Yamaguchi 1967; Halperin 1964). However, in *Santalum* it is rather difficult to specify a particular factor causing this phenomenon.

The embryogenic potentiality of *Santalum* cultures did not decline during serial subcultures as is observed in many tissues (Narayanaswamy 1977). This is of considerable interest because it affords a possibility to harvest young sandalwood plantlets from differentiated embryos continuously.

The asexually derived embryos in *Santalum* if kept without harvesting produced callus, which in turn could regenerate multitudes of embryos. Thus the differentiation of embryos was a continuous process.

The results presented here and those briefly reported earlier (Rao and Bapat 1978; Bapat and Rao 1979) point to the standardisation of the technique for obtaining plant regeneration through organogenesis and embryogenesis in the cultured hypocotyl explants of *Santalum album*, which may have some use in the multiplication of the species in which vegetative propagation has not met with desired success.

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A contribution to the embryology of *Ctenolepis garcini*

H MAHESWARI DEVI and K CHANDRASEKHARA NAIDU
Department of Botany, Andhra University, Waltair 530 003, India

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Abstract. Embryology of *Ctenolepis garcini* Hook. was studied in detail and is described and illustrated.

Keywords. Embryology; *Ctenolepis garcini*; Trichosantheae; Cucurbitaceae.

1. Introduction

Ctenolepis garcini Hook. (= *Blastania garcini*) belongs to the tribe trichosantheae of the family cucurbitaceae. The tribe comprising 10 genera and 120 species (Jeffery 1962) is mostly confined to the warmer parts of the globe. All the previous reports in this tribe are fragmentary and not even in a single species, detailed account is given except in *Trichosanthes dioica* (Banerji and Das 1937; Singh 1956) and *Trichosanthes anguina* (Chopra 1955; Singh 1956). Singh (1964, 1967) studied the seed coat and endosperm in *Herpetospermum*, *Edgaria*, *Biswarea* and *Ctenolepis ceraciformis*. Singh and Dathan (1976) reported the seed coat structure coat in eight species of *Trichosanthes*. It was therefore proposed to study the embryology of *C. garcini*.

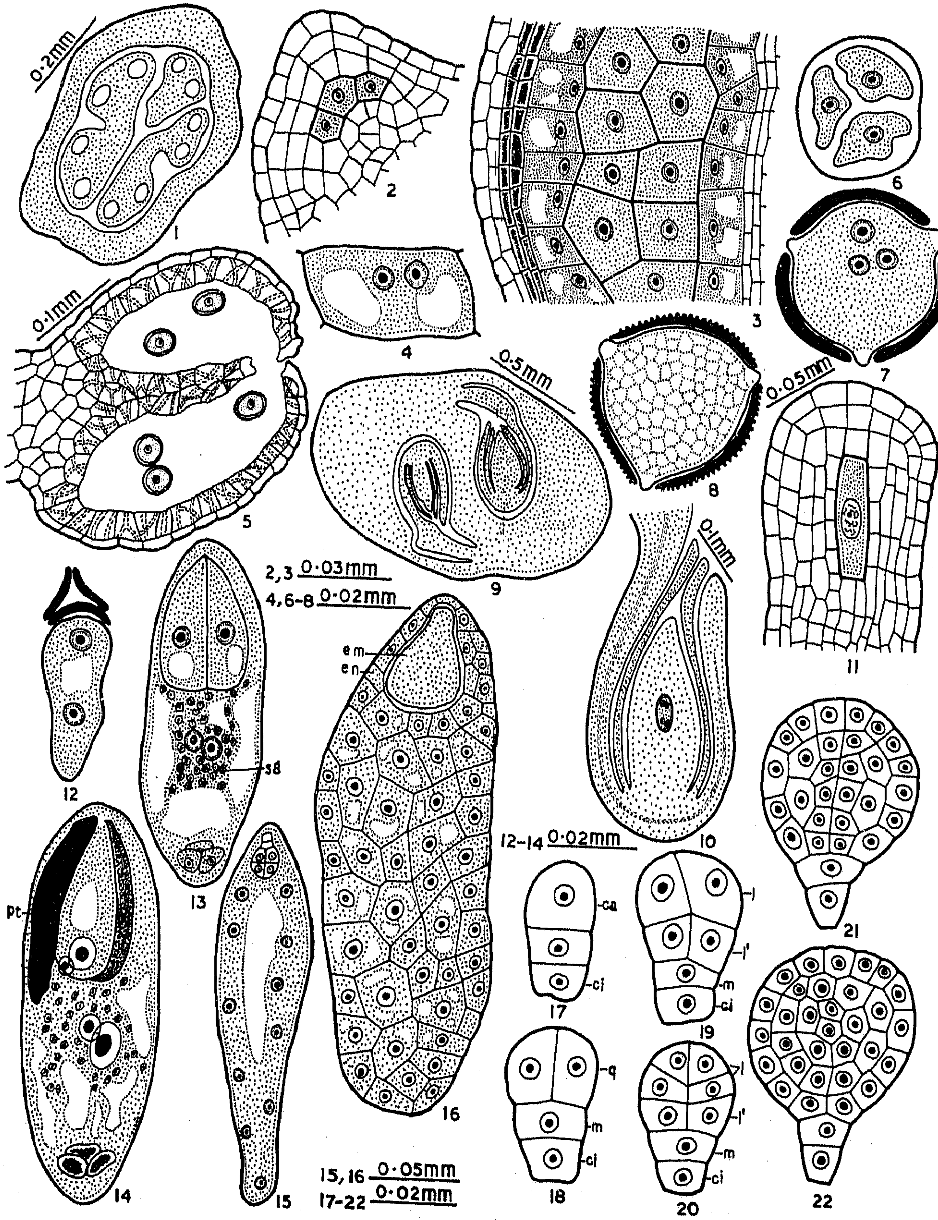
2. Material and methods

The material collected from Botanical garden of Andhra University was fixed in Formalin-Acetic-Alcohol (FAA). Customary methods of dehydration, infiltration and embedding were followed (Johansen 1940). Sections, 5–15 μ thickness, were stained in Delafield's haematoxylin.

3. Observations

3.1 *Microsporangium, microsporogenesis and male gametophyte*

Of the three anthers two are tetrasporangiate and one is bisporangiate (figure 1). The hypodermal archesporium is 2 or 3-celled in TS in each lobe. It undergoes a periclinal division and produces an outer primary parietal layer and an inner primary sporogenous layer. The former divides periclinally to produce an inner tapetal layer and an outer secondary parietal layer (figure 2) which as a result of one more periclinal division gives rise to an outer endothecial layer and an inner middle layer. The middle layer undergoes one periclinal division forming two middle layers (figure 3). Thus, the development of another wall follows the Dicotyledonous type. The secretory tapetum is uniseriate with binucleate cells (figure 4). Endothecial cells develop fibrous



Figures. 1–22. **1.** TS of male flower. **2.** TS of a part of anther lobe showing development of wall layers and sporogenous tissue. **3.** LS of a part of anther lobe showing sporogenous tissue and wall layers. Note degenerating middle layers. **4.** Tapetal cell. **5.** TS of mature anther lobe showing fibrous endothecium and uninucleate pollen grains. **6.** Pollen tetrad. **7, 8.** Pollen grains. **9.** TS of bicarpellery ovary. **10.** LS of ovule. **11.** Nucellus showing parietal cells with megaspore mother cell. **12, 13.** Development of embryo sac. Note starch grains in 13. **14.** Embryo sac showing fertilisation. **15, 16.** Stages in the development of endosperm. **17–22.** Stages in the development of embryo. (*em*: embryo; *en*: endosperm; *pt*; pollen tube; *sg*: starch grains).

ickenings and middle layers become crushed as the anther matures (figure 5).

The primary sporogenous cells after undergoing one or two mitotic divisions evelop into pollen mother cells. They undergo simultaneous meiotic divisions to roduce tetrahedral tetrads (figure 6). Cytokinesis is by furrowing. Pollen grains e tricolporate and are shed at 3-celled stage. The exine is thick and ornamented gures 7, 8).

2 *Ovary, ovule, megasporogenesis and female gametophyte*

he ovary is inferior, bicarpellary syncarpous and unilocular with parietal placentae gure 9). The ovule is anatropous, bitegmic and crassinucellate. The micropyle is rmed by the inner integument. The funicle has a single vascular strand (figure 10). he hypodermal archesporium is single-celled. It divides periclinally and produces a rimary parietal cell and a megaspore mother cell. The former divides both by anti and ericlinal divisions (figure 11) giving rise to several-layered parietal tissue above the egaspore mother cell making it deep seated. The parietal tissue together with the ucellus forms a prominent nucellar beak which extends into the micropyle (figure 10). he nucellus remains persistent up to the early heart-shaped embryo stage. The egaspore mother cell elongates considerably and undergoes meiotic divisions to roduce a *T*-shaped tetrad (figure 12). The functional chalazal megaspore after ndergoing three mitotic divisions produces an 8-nucleate embryo sac of the olygonum type (figures 12, 13). The synergids are pear-shaped. The three antipodals re uninucleate and ephemeral. Starch grains are present in the centre of the embryo c (figure 13).

3 *Fertilisation and endosperm*

he path of the pollen tube is porogamous. The pollen tube on its way into the embryo c crushes the cells of the inner integument and the cells of nucellar beak creating a ide space. At this region the pollen tube dilates considerably. Triple fusion and ngamy occur more or less simultaneously (figure 14). The development of endosperm of the Nuclear type. After a few free nuclear divisions the endosperm nuclei become istributed along the periphery enclosing a central vacuole (figure 15). Cell wall rmation commences at the micropylar region and proceeds towards the chalazal gion, ultimately filling the entire embryo sac with cellular tissue (figure 16). The ndosperm is completely consumed by the growing embryo.

4 *Embryo*

he zygote undergoes a transverse division resulting in a 2-celled proembryo. The asal cell *cb* divides transversely producing two superposed cells *m* and *ci* (figure 17). he terminal cell *ca* next divides vertically and a 4-celled *T*-shaped proembryo is rmed (figure 18). The two apical cells of the proembryo undergo one more vertical vision to engender quadrant *q* which divides by a transverse wall forming an octant two tiers each consisting of 4 cells (figure 19). They are termed as *l* and *l′*. Due to ericlinal divisions in the tiers *l* and *l′* dermatogen initials are differentiated towards eriphery. The cells of inner layer undergo further divisions in all planes, giving rise to e pleurome and periblem initials (figures 20–22). The derivatives of the tier *l* ontribute to the formation of the cotyledons and stem tip and those of *l′* contribute to

the hypocotyledonary region and root. The cells *m* and *ci* remain undivided and contribute to the suspensor (figures 21, 22). The mature embryo is straight with two leafy cotyledons (figure 23).

From the above it is clear that the four-celled proembryo is *T*-shaped and the derivatives of *ca* alone contribute to the embryo proper. The two cells *m* and *ci* directly develop into a two-celled suspensor. Thus the embryo development conforms to the Onagrad type.

3.5 *Development of seed coat*

The seed coat is formed by the outer integument alone. The inner integument shows signs of degeneration with the entry of pollen tube into the micropyle and by the time a globular embryo is formed no traces of the inner integument are left (figures 24–28).

At the functional embryo sac stage the outer epidermis of the outer integument divides periclinally producing two layers of cells of which the outer one again undergoes a periclinal division and thus three layers of cells e, e_2 and e_1 result (figures 25–27). Of the three layers the outermost layer e functions as the seed coat epidermis and the innermost layer e_1 becomes sclerenchymatous (figure 29).

The layer e_2 by repeated periclinal and a few anticlinal divisions contributes to the seed coat hypodermis. At six-celled embryo stage the hypodermis is parenchymatous, containing three or four layers of cells (figure 28). By the time a mature embryo is formed the parenchymatous hypodermis becomes nine-layered sclerenchymatous zone (figure 30). Meanwhile the parenchymatous tissue of the middle zone of the outer integument gradually elongates and a few of its cells show signs of degeneration. Ultimately, four or five layers of cells remain healthy and participate in the formation of the seed coat (figure 30). Simultaneously the epidermis of the seed coat again undergoes one more periclinal division and produces two layers (figure 30) which also contribute to the formation of the seed coat.

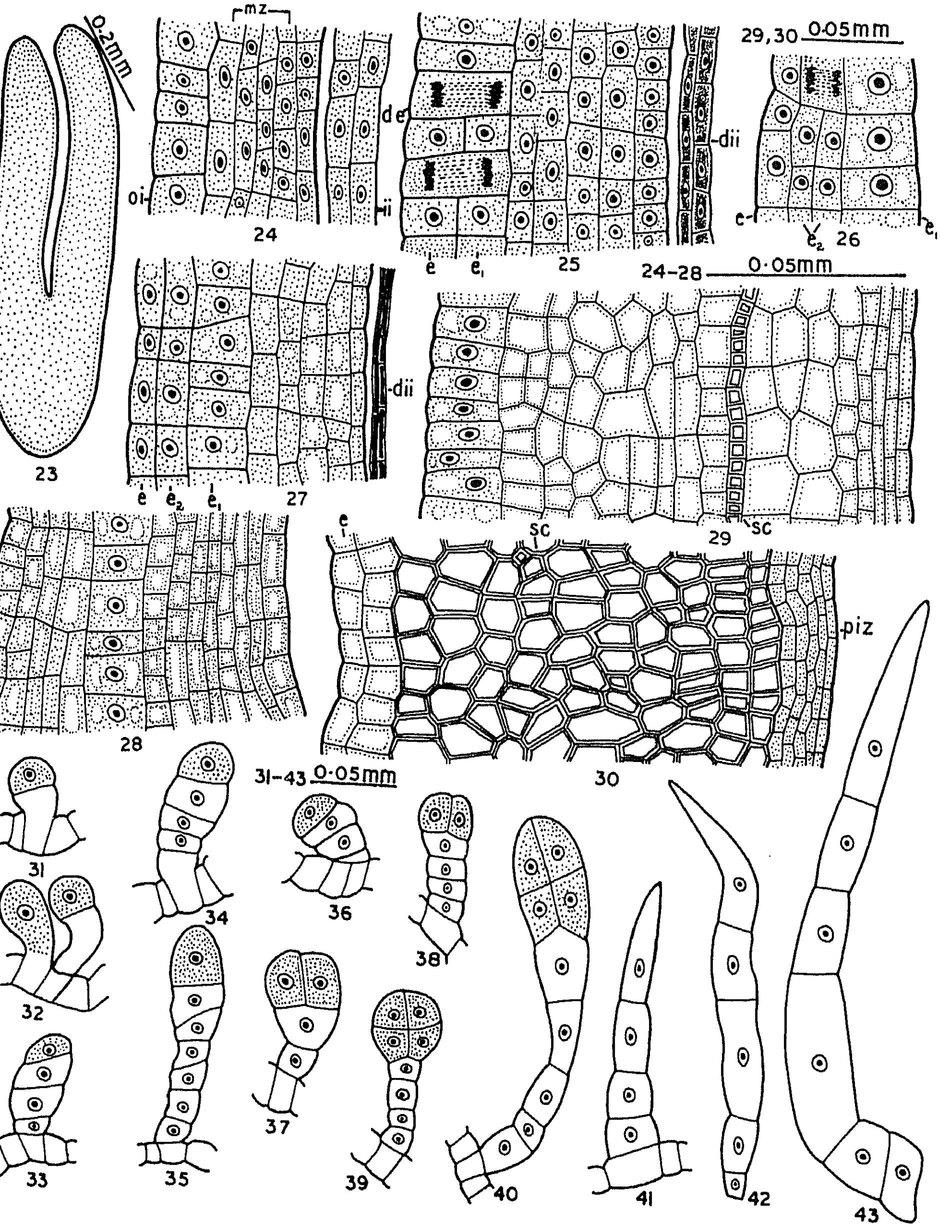
Thus the seed coat is formed by the outer integument alone and the mature seed coat contains two layers of epidermis, sclerenchymatous hypodermis and parenchymatous inner zone.

3.6 *Structure and ontogeny of trichomes*

Both glandular and non-glandular trichomes have been observed on the staminate and pistillate flowers of the plant investigated.

3.6a *Glandular trichomes*: Any epidermal cell may function as the hair initial. It elongates and accumulates abundant cytoplasm and undergoes a transverse division to give rise to a basal cell and an apical cell. The apical cell again divides transversely to form a stalk cell and a head cell (figure 31). The basal cell does not undergo further divisions and functions directly as the foot. The stalk cell either remains undivided and forms a short stalk or undergoes several transverse divisions to produce a long uniseriate stalk (figures 32–35). The head cell either directly functions as a single-celled head or divides to give a multicellular head (figures 34–40).

3.6b *Non-glandular trichomes*: These follow the same sequence of development as the glandular trichomes. However, the head cell becomes pointed (figures 41–43).



Figures. 23–43. 23. Mature embryo. **24–30.** Stages in the development of seed coat. **31–43.** Trichomes. (*de*: dividing epidermis; *dii*: degenerating inner integument; *ii*: inner integument; *oi*: outer integument; *sc*: sclerenchyma. *mz*: middle zone).

Discussion

structure and development of anther wall in the present investigation resembles t in *T. dioica* (Banerji and Das 1937) and *T. anguina* (Singh 1956). Although there two middle layers, the development of the anther wall in *C. garcini* is not of the

basic type, as the second middle layer is formed as a result of periclinal division of the original middle layer and hence the development of anther wall with a slight deviation is of the Dicotyledonous type (Davis 1966). The female archesporium in *C. garcini* is single-celled, but it is multicellular in *T. dioica* (Banerji and Das 1937). However, single megaspore mother cell is differentiated in all the hitherto investigated species.

The Nuclear type of endosperm development and formation of chalazal endosperm haustoria are common in the taxa of this tribe except *C. garcini* (Chopra 1954, 1955; Chopra and Seth 1977; Singh 1964, 1967; present study).

So far there is no report on the development of embryo in the tribe Trichosantheae. In the present study the development of embryo is traced and it conforms to the Onagrad type. In *C. garcini* the seed coat is formed by the outer integument alone and follows the usual Cucurbitaceous pattern as in the species of *Trichosanthes*, *Herpetospermum*, *Edgaria* and *Biswarea* (Singh 1967; Singh and Dathan 1976).

Acknowledgements

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SEM studies on seed surface of wild and cultivated species of *Vigna* Savi

DINESH KUMAR and N S RANGASWAMY
Department of Botany, University of Delhi, Delhi 110 007, India

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Abstract. Scanning electron microphotographs of seed surface patterns of three wild and five cultivated species of *Vigna* are provided and described. The seed surface patterns are species-specific and showed no interpopulation variation. To promote the use of technical terms in SEM studies of seed surface and to serve as a ready reference a table explaining the 13 terms adopted by us is given. The importance of study of seed surface especially in taxonomy is discussed.

Keywords. Seed surface; *Vigna*; Leguminosae; wild populations; cultivated species; scanning electron microscopy

1. Introduction

Vigna Savi is one of the pulse-yielding genera. Of 100–150 species which the genus comprises, 20 are native to India. *V. angularis*, *V. radiata*, *V. sinensis*, *V. umbellata* and *V. unguiculata* are cultivated species; *V. aconitifolia* is found in both cultivated and wild states. The remaining 14 species occur wild.

Generally the wild relatives of crop plants are adapted to greater variations in climatic and edaphic conditions than are the crop plants. Therefore the wild relatives constitute an invaluable gene pool of natural variations (Boerma 1970; Zohary 1970). However, studies on the wild relatives of pulse crops are meagre; *Vigna* Savi is no exception to this.

In his studies of Papilionoideae Verdcourt (1970) has stated that "... the whole matter [of taxonomy of *Vigna*] is very confused and no sound decisions can [we] come to until a great deal of work has been done ...." Further, the exhaustive tabulation by Brisson and Peterson (1976, 1977) on scanning electron microscopy (SEM) of seeds shows that of over 700 angiosperms studied only one species is of *Vigna* (Nwanze *et al* 1975). Later studies on seed coats of some four species of *Vigna* (Rajendra *et al* 1979; Jain and Babu 1982), on those of *Phaseolus* spp. (Sharma *et al* 1977, 1982), on those of the tribe Vicieae (Trivedi *et al* 1978; Lersten 1979), and on those of some Mimosoideae and *Bauhinia* spp. (Trivedi *et al* 1979, 1980) reaffirm the value of SEM studies as a reliable tool in elucidating taxonomic and genetic relationships. Recognizing this, we undertook SEM studies on seed surface patterns of three wild and five cultivated species of *Vigna*.

2. Materials and methods

Seeds of 12 populations of *Vigna* species were studied (table 1). From each population, mature, naturally dried seeds collected at random from several individuals were

Table 1. Seed collection data on species of *Vigna* Savi.

Species*	Population code	Place of collection
Wild species		
V. aconitifolia (Jacq.) Marech.	AO I	Rocky habitat near Hirakud Dam, Sambalpur Distt, Orissa
	AO II	Railway embankments, Hirakud, Sambalpur Distt, Orissa
V. trilobata (L.) Verd.	TM I	Borai, Sitanadi Range, MP
	TM II	Bachi Gaon, Sitanadi Range, MP
V. vexillata (L.) A. Rich.	VB I	Ranchi, Bihar
	VM II	Chotta Donger, Bastar Distt, MP
Cultivated species		
V. aconitifolia (Jacq.) Marech. cv Moth PLMO-21	VA I	Univ. Delhi (seeds originally from Indian Agricultural Research Institute, New Delhi)
V. ricciardiana (Tenore) Babu*	RB I	Netrahat, Bihar
V. sinensis (L.) Hassk.	SSM I	Chotta Donger, Bastar Distt, MP
	SSM III	Gura Bera, Bastar Distt, MP
V. umbellata (Thunb.) Ohwi & Ohashi cv NBPGR-1	VUM I	Indian Agricultural Research Institute New Delhi
V. unguiculata (L.) Walp var. V-16	VUN I	

* The circumscriptions of all the species except *V. ricciardiana* are after Verdcourt (1970).

studied. The untreated seeds (figure 1A) were cleared ultrasonically for 10 min and mounted with a silver paste along the circumference of the specimen disc with the hilum laterally anticlockwise and toward the circumference of the disc (figure 1B). The mounted seeds were gold-coated (500 A) in an Edward's Vacuum Evaporation Unit and the free surface adjacent to the hilum (figure 1B) was scanned at a constant angle of 45° at an accelerating potential of 10 kV in a $S_4$10 Model SEM (Cambridge). The microphotographs were taken between 265 × and 7400 ×.

To understand the broad chemical nature of the seed surface, mature dry seeds of only *V. aconitifolia* were treated with (i) 70 % ethanolic solution of Sudan III, (ii) cold concentrated H_2SO_4 for 2 min, (iii) cold concentrated H_2SO_4 for 2 min followed by IKI reagent, (iv) Sudan Black B, (v) chlor-zinc iodide, (vi) cold chloroform, and (vii) boiling chloroform.

Most of the earlier SEM studies on seed surface have described the seed surface patterns in non-technical terms or in comparison to animal or vegetable specimens such as octopus, sea anemone, and cauliflower. We have, however, adopted the technical terms used by Murley (1951, see Stearn 1966) for describing the seed surface patterns revealed through optical microscopy (table 2).

3. Observations

3.1 *Wild species*

3.1a *Vigna aconitifolia*: The seed surface is scrobiculate in both the wild populations studied (figure 1C–F). In whole seeds treated with an alcoholic solution of Sudan III

the surface stained red. From water-soaked seeds a discrete covering could easily be removed; it presents a compartmentalized pattern. The surface covering disintegrates in cold concentrated sulphuric acid. All the other chemical tests with whole seeds as well as with the removed covering showed the latter to be largely waxy. Optical sections of both sulphuric acid-treated seeds and seeds from which the surface covering had been mechanically removed showed exposed palisade layer.

3.1b *Vigna trilobata*: In both the populations studied the seed surface is reticulate-foveate to reticulate (figure 1G, H, J). The reticulum is presumably waxy and the wax deposit is rather thick at the junction points. In the interspaces of the reticulum a network of polygonal areas which in turn are made of a finer network are seen in relief (figure 1H, J). This structure in relief is more discrete in the population TM I than in TM II; in all other aspects the seed surface pattern in both the populations is identical.

3.1c *Vigna vexillata*: The seed surface is only apparently reticulate; a closer examination shows a scalariform pattern (figure 1K, L). However, owing to compression of the spaces between the crossbands, the overall pattern can be best considered intermediate to reticulate and scalariform. The surface deposition was more in the population VM II than in VB I; nevertheless, no interpopulation variation could be observed.

Thus in each of the three wild species studied the microsculpture of seed surface is distinct.

3.2 *Cultivated species*

3.2a *Vigna aconitifolia*: In contradistinction to the seed of wild population, that of the cultivated population shows a pusticulate surface (figure 2A). The arrangement of the pustules is only apparently reticulate. Underlying the pusticulate surface and seen in relief is a cord-like and at places a granular matrix. The similar appearances of the surface and the matrix in electron microphotographs (figure 2B–D) permit the assumption that the two are chemically identical. Except for some micro-variation in the outline of the pustules, all seeds examined showed a pusticulate surface (figure 2B–D).

Also, in contrast to the seed surface of the wild population, that of the cultivated population gave only a mild reaction for waxes and fats. Thirdly, upon water-soaking, the seeds of the cultivated population did not yield any detachable covering.

3.2b *Vigna ricciardiana*: All the seeds scanned showed a glebulate-ruminate surface (figure 2E, F).

3.2c *Vigna sinensis*: The seed surface is favulariate and shows some microvariations (figure 2G–J).

3.2d *Vigna umbellata*: The seeds scanned showed a ruminate surface (figure 2K–M), and seldom a falsifoveate to foveolate surface (figure 2N, P).

3.2e *Vigna unguiculata*: All seeds studied showed a glebulate surface (figure 2Q, R).

In all the species studied the seed surface pattern is species-specific. No intrapopulation variation (except in *V. umbellata*), nor any interpopulation variation could be discovered; however, the wild and the cultivated populations of *V. aconitifolia* showed differences in their seed coat surface patterns.

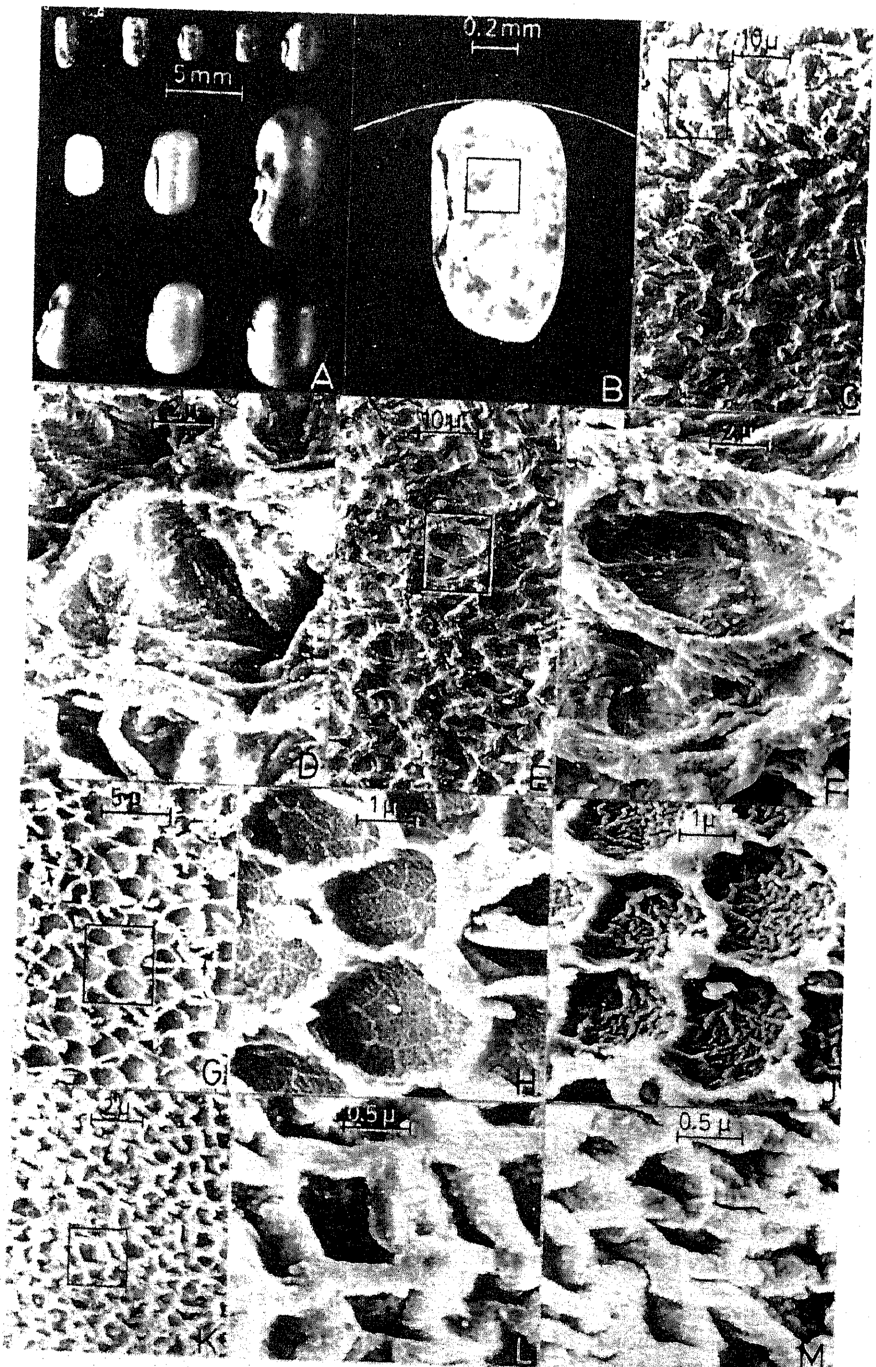
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Table 2. Technical terms* adopted to describe seed surface patterns.

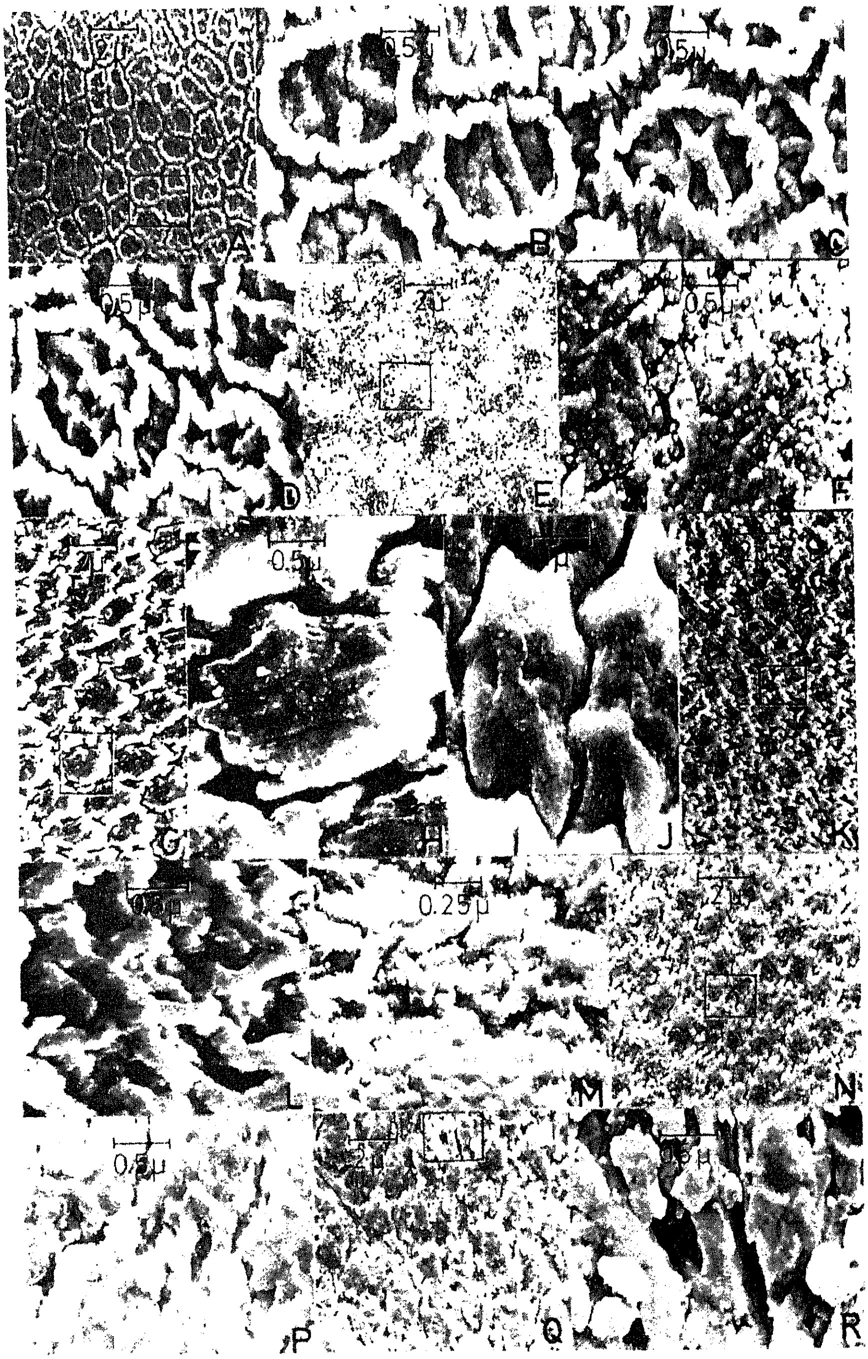
Term	Explanation
Colliculate	Having rounded broad elevations closely spaced covering the seed coat
Falsifoveate	Having pits that do not have uniform depth, little depressions made laterally
Favulariate	Finely ribbed, the ribs separated by zig-zag furrows
Foveate	Pitted or having depressions
Foveolate	Marked with little pits
Glebulate	Having small clumps of randomly placed granules
Pusticulate	Having small broad elevations not so high or abundant as on colliculate surface and not having as abrupt elevations as a minutely tuberculate surface
Reticulate	Having a raised net work of narrow and sharply angled lines frequently presenting a geometric appearance, each area outlined by reticulum being an interspace
Reticulate-foveate	A type in between reticulate and foveate types
Ruminate	Penetrated by irregular channels giving an eroded appearance and running in different directions
Scalariform	Having small fairly regular cross bands suggestive of the rungs of a ladder
Scrobiculate	Having elongated shallow depressions or pits
Tuberculate	Having small smooth rounded projections or knobs

* From Stearn (1966).

4. Discussion

None of the seed surface patterns observed by us resembles that of any other four species or of the two interspecific hybrids of *Vigna* illustrated so far (Rajendra *et al* 1979; Jain and Babu 1982). The seed surface pattern of *V. ricciardiana* needs a special mention. According to Verdcourt (1970) *Phaseolus ricciardianus* Tenore is a synonym of *V. umbellata* (Thunb.) Ohwi and Ohashi. However, the unpublished observations of

Figures 1A–M. **A.** One representative seed each of 11 of the 12 populations of *Vigna* species studied. Left to right, **top row**: *V. aconitifolia* wild populations AO I and AO II, *V. trilobata* populations TM I and TM II, and *V. vexillata* population VM II; **middle row**: *V. aconitifolia* cultivated population VA I, *V. ricciardiana* population RB I, and *V. sinensis* population SSM I; **bottom row**: *V. sinensis* population SSM III, *V. umbellata* population VUM I, and *V. unguiculata* population VUN I. **B.** Seed showing its orientation on specimen stub and the region of its surface that was scanned. White line in continuity with seed margin represents an arc of the specimen stub and the enblocked area the seed surface scanned. **C–F.** SEM graphs of surface of untreated seeds of wild populations AO I (**C, D**) & AO II (**E, F**) of *V. aconitifolia*. The seed surface is scrobiculate showing shallow depressions. **D, F** are magnified views of regions shown enblocked in **C** and **E** respectively. Contrast with **2A–D.** **G–J.** *V. trilobata* populations TM I (**G, H**) and TM II (**J**). Seed surface shows a reticulate-foveate to reticulate pattern. Reticulation is distinct at places; at other regions (arrows in **G**) it shows little pits (foveolate). **H.** Magnified view of region enblocked in **G.** **K–M.** *V. vexillata*; the underlying scalariform pattern is distinct in **L** which is the magnified view of region enblocked in **K. K, L** are of population VM II from MP and **M** is of population VB I from Bihar.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Dr C R Babu (University of Delhi) show that the pod characters of *P. ricciardianus* are so distinct that it deserves to be treated as a species of *Vigna* (following Verdcourt's circumscription of the two genera *Vigna* and *Phaseolus*), namely *V. ricciardiana* (Tenore) Babu (*cf.* footnote in table 1). Interestingly our SEM studies have shown that the glebulate-ruminate seed surface of *V. ricciardiana* bears some resemblance to the ruminate seed surface of *V. umbellata*. However, *V. umbellata* itself has exhibited intrapopulation variation. Therefore, the taxonomic identity of *P. ricciardianus vis-a-vis V. umbellata* and *V. ricciardiana* is still an open area for research. Likewise, the intrapopulation variation in *V. umbellata* also calls for further delimitation of the taxon into infraspecific categories.

Lersten (1979) observed the seed surface patterns in Vicieae to be distinctive from those of nearly 200 other genera of Papilionoideae. Like the work on eight species of Viciae (Trivedi *et al* 1978) our work has also shown the seed surface to be species-specific. The work of Nwanze *et al* (1975) has demonstrated that the seed surface patterns help distinguish varieties as well. SEM studies on *Vigna sinensis* and *V. sesquipedalis* and their hybrids have shown the seed surface patterns to be genetically controlled (Rajendra *et al* 1979). All these investigations including ours clearly prove that seed surface pattern is a conservative trait and can therefore be of great value in taxonomic delimitations and in hybridization as a genetic marker.

In our study the presence of a mechanically detachable thick covering on seeds of the wild populations of *V. aconitifolia* in contradistinction to those of a cultivated population of the same species is of interest. In *Glycine* of Phaseoleae (to which *Vigna* also belongs) remnants of the inner pod wall are known to be adherent to the seed. Ontogenetic studies are needed to establish whether or not the thick surface covering on seeds of the wild *V. aconitifolia* is *pro parte* the pericarp. Chemical tests have proved the waxy nature of this covering. The absence of such a waxy covering in the cultivated population of *V. aconitifolia* may indicate an adaptation to domestication in that it increases the permeability of seed coat to water enabling uniform germination under human care.

In *V. unguiculata* (cow pea) bruchid larvae penetrate the smooth seed coat varieties in greater numbers than they do the rough seed coat varieties (Nwanze and Horder 1976); in bruchid-resistant varieties of *Cicer arietinum* (chick pea) the seeds have a

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Figures 2A–R. All are SEM graphs of surface of untreated seeds of *Vigna* species. **A–D** are of cultivated population of *V. aconitifolia*. Surface is pusticulate; closer examination falsifies the reticulate pattern. Contrast with surface of seeds of wild populations of *V. aconitifolia* (figure **1C–F**). **B.** Magnified view of region enblocked in **A. B** and **C** show granular region of matrix in relief. **B, C, D** are from different individuals. **E, F.** *V. ricciardiana*; the seed surface is glebulate-ruminate. **F.** Magnified view of region enblocked in **E**; compare with the patterns in **P** and **R. G–J.** *V. sinensis*; **G** shows general view of the favulariate pattern of seed surface, and **H** a magnified view of region enblocked in **G.** Both are from population SSM I from MP. **J** is magnified view of surface of seed from population SSM III, also from MP. Except for a microvariation in the contour of ribs and furrows the overall pattern is identical with that in **H. K–P.** *V. umbellata*; **K–M** show ruminate pattern. **L** is magnified view of region enblocked in **K, M** is of another seed. Pattern in **L** superficially resembles that in **1 L, M** (*V. vexillata*). **N, P.** Falsifoveolate-foveolate pattern. **P** is magnified view of region enblocked in **N. Q, R.** *V. unguiculata*; general view and enlarged view of seed surface respectively. Note the glebulate pattern.

rough surface which acts as a deterrent to oviposition by bruchids (Schalk *et al* 1973). These observations further point to the applied value of SEM studies on seed surface.

Indeed, Heywood's (1971) remark that SEM studies of seed coat will become a routine in studies of seed biology aptly concludes this discussion.

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Reproductive morphology of *Hoppea fastigiata* C B Clarke

K SANKARA RAO*
Department of Biology, The University of Calgary, Calgary, Alberta, Canada T2N 1N4
* Present address: Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India

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Abstract. The reproductive morphology of *Hoppea fastigiata* has been studied and described. Evolutionary trends in the subtribe Erythraeinae are highlighted.

Keywords. *Hoppea fastigiata*; reproductive morphology; Gentianaceae.

1. Introduction

Although Gentianaceae have been the subject for many morphological and developmental studies, relatively little information is available on the subtribe Erythraeinae members of which are of considerable ecological and morphological interest. Studies so far carried out encompass the halophytic *Enicostemma littorale* (Srinivasan 1941), saprophytic *Obolaria virginica* (Johow 1885; Holm 1897), xerophytic *Cicendia filiformis* (Guérin 1926) and a few mesophytic taxa of mostly grassland vegetation viz., *Erythraea centaurium* (Stolt 1921; Guérin 1926; Crété 1949a, b), *Chlora perfoliata* (Crété 1955), *Hoppea dichotoma* (Arekal 1961; Sankara Rao 1978), *Canscora diffusa, C. decussata* (Maheswari Devi 1962), *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969), *Erythraea roxburghii* (Maheswari Devi and Satyanarayana 1971), *Canscora decurrens* (Maheswari Devi and Lakshminarayana 1977; Sankara Rao 1979). In planning a further study of the reproductive morphology of Erythraeinae, *Hoppea fastigiata* C. B. Clarke, a hygrophytic species endemic to India has been selected (Gamble 1928). Features of evolutionary significance in Erythraeinae have been summarised in the light of available data.

2. Material and methods

Hoppea fastigiata C. B. Clarke, a tiny herb with quadrangular stem, opposite small sessile leaves and pale yellow flowers, was collected from the National Park, Bannerghatta, Karnataka state. Voucher specimens were deposited with the herbarium, Centre for Taxonomic Studies, Bangalore (JCB). Flower buds and fruits in different stages of development were fixed in formalin-acetic -ethanol, dehydrated and embedded from an n-butanol series. Paraffin sections, 7–12 μm thickness, were stained in haematoxylin, and counter-stained in erythrosin. Sections of mature seeds were stained separately with bromophenol blue, periodic acid-Schiff's reagent and sudan black-B to determine the nature of stored food in the endosperm (Jensen 1962).

3. Observations

3.1 *Microsporangium*

Flowers are protandrous with a single fertile stamen. The anther is tetrasporangiate. The development of the microsporangial wall follows the dicotyledonous sequence (figures 1–4). The cells of the sporogenous layer undergo division and enlargement and function as microsporocytes (figures 2–4). Part of the tapetum bordering the inner face of the sporogenous tissué is derived from the ground tissue near the connective and becomes continuous with the tapetum on the outer face (figures 3, 4). Thus, the tapetum is of dual origin. The tapetal cells remain uninucleate and become vacuolate at the time of microsporogenesis (figures 3, 4). They undergo radial elongation and protrude into the anther locule, especially from the connective side (figure 4). Following microsporogenesis, the glandular tapetum degenerates.

In the mature anther, the epidermis is persistent and the endothecium is fibrillar. The middle layer is crushed during the development of anther.

3.2 *Microsporogenesis and male gametophyte*

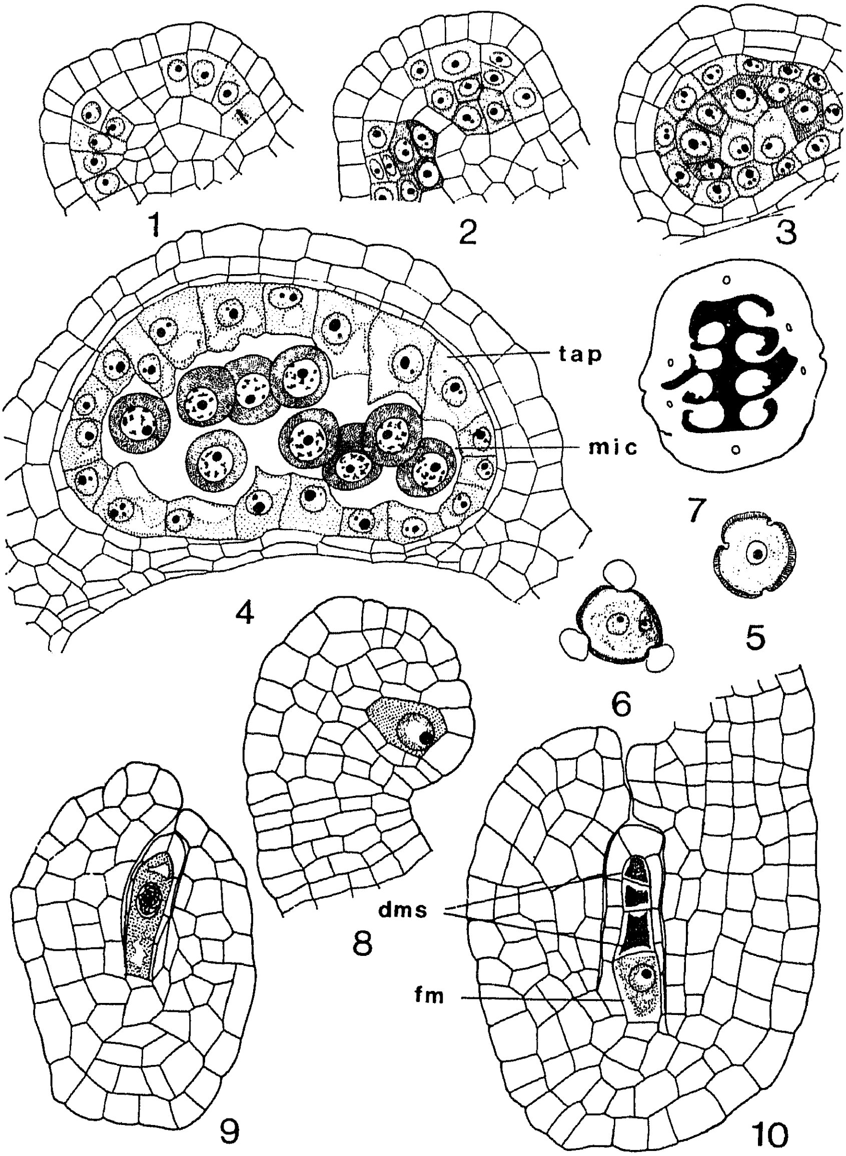
The microsporocytes by meiosis and simultaneous cytokinesis produce tetrahedral microspore tetrads. The microspores separate from the tetrad when they are uninucleate (figure 5). The division of microspore nucleus results in a male gametophyte with a large vegetative cell and smaller lenticular generative cell. At about this time, certain cytoplasmic hyaline capitate processes, one at each germ pore region appear on the pollen grain and fall off before anther dehiscence (figure 6). Pollen grains are isopolar, 3-zonocolporate and oblate-spheroidal ($30 \times 34\,\mu$m). Exine surface is striato-reticulate.

3.3 *Megasporogenesis and female gametophyte*

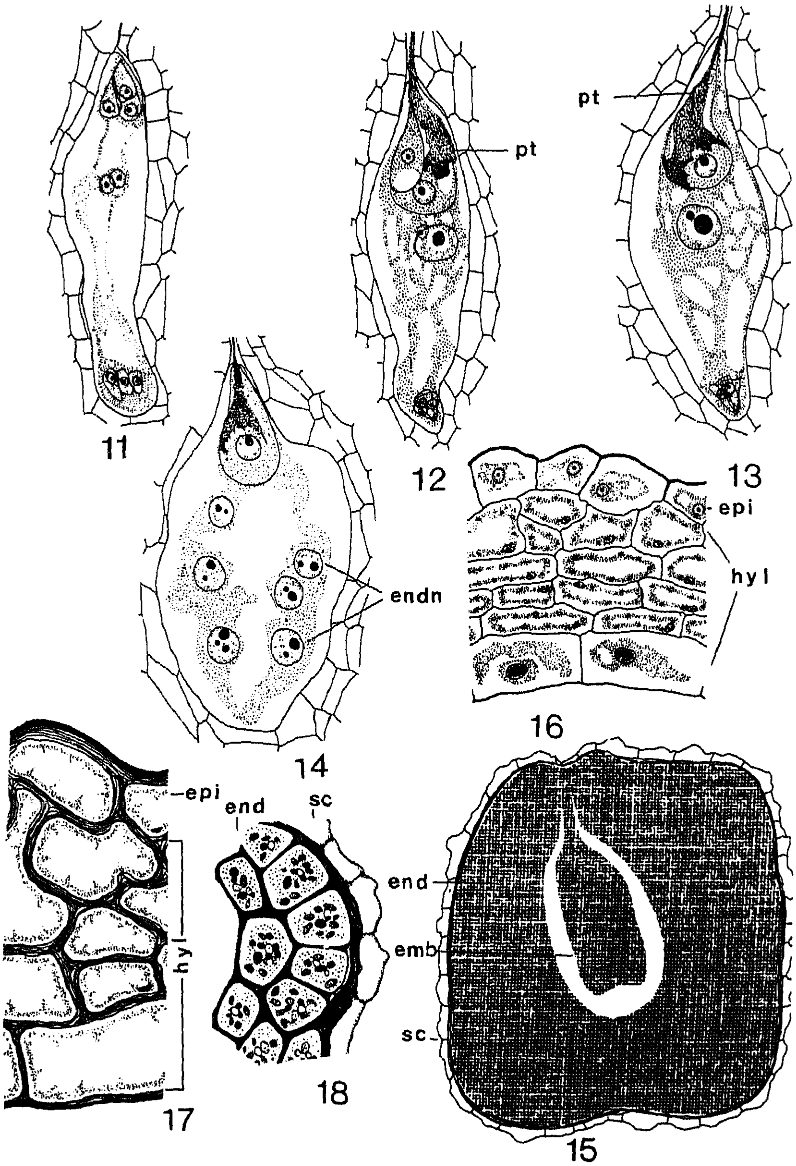
The two placentae are marginal and forked at the base (figure 7). The ovules are numerous, anatropous, tenuinucellar, unitegmic and borne in vertical rows on the placentae (figures 7–10). They develop as small protuberances and start curving at an early stage. Growth of the integument around the nucellus is complete shortly after the megasporocyte is formed in the nucellus (figure 9). In the mature ovule, the integument is five or six cells thick and the micropyle is long and narrow (figure 10).

The hypodermal archesporium functions directly as the megasporocyte (figures 8, 9). Through meiosis, the megasporocyte forms a linear tetrad of megaspores. The functional chalazal megaspore in the tetrad undergoes three successive free-nuclear divisions producing an eight-nucleate female gametophyte of the Polygonum type. The eight nuclei in the gametophyte organise themselves into an egg apparatus at the micropylar end, three antipodals at the chalazal end and two polar nuclei (figure 11). In the mature female gametophyte which is more or less spindle-shaped, the two synergids as well as the large egg are pear-shaped. The polar nuclei fuse at the center and the resulting secondary nucleus moves close to the egg apparatus. The antipodals are organized into cells with their pointed ends directed towards the chalaza. These cells remain persistent till the endosperm becomes two-nucleate (figures 12, 13). During the early phase of female gametophyte formation, the epidermal cells of the nucellus are crushed and absorbed.

The entry of the pollen tube is porogamous and enters the gametophyte through one



Figures 1–10. 1. TS of young anther showing the archesporial primary parietal and primary sporogenous cells × 600. **2–3.** TS of young anther lobes showing wall layers and sporogenous cells × 600. **4.** TS of anther lobe showing wall layers and microsporocytes × 600. **5.** Microspore × 800. **6.** Two-celled pollen grain × 800. **7.** TS of ovary × 100. **8.** L S young nucellus showing hypodermal archesporial cell × 600. **9.** L S young ovule showing megasporocyte × 600. **10.** L S ovule showing functional megaspore × 600. (*fm*, functional megaspore; *dms*, degenerating megaspores; *mic*, microsporocytes; *tap*, tapetum).



Figures 11–18. **11.** Organized female gametophyte × 600. **12–13.** Stages showing fertilization of female gametophyte × 600. **14.** Zygote and nuclear endosperm × 600. **15.** L S seed (outline) × 120. **16.** Cross-section of ovary wall × 250. **17.** Cross-section of pericarp × 250. **18.** Cross-section of seed coat × 250. (*emb*, embryo; *end*, endosperm; *endn*, endosperm nuclei; *epi*, epidermis; *hyl*, hypodermal layers; *pt*, pollen tube; *sc*, seed coat).

of the synergids and discharges its contents. Double fertilization occurs. The synergids degenerate soon after fertilization (figures 12, 13).

3.4 *Endosperm*

The development of the endosperm is the Nuclear type. The division of the primary

endosperm nucleus precedes that of the zygote. By a series of successive divisions, a large number of free nuclei are formed (figure 14). With increase in number of nuclei in the endosperm, the central vacuole breaks up into several smaller ones. This is followed by an increase in density of the cytoplasm in which the nuclei remain uniformly distributed. Cell walls are laid in the cytoplasm simultaneously at this stage. Only a part of the endosperm tissue is consumed by the developing embryo and therefore, the mature seed is endospermic with protein crystals as reserve food (figures 15, 18).

3.5 *Embryogeny*

The zygote divides transversely to form a terminal cell, *ca* and a basal cell, *cb* (figures 19, 20). The terminal cell *ca* in this two-celled proembryo divides transversely to form two cells, *cc* and *cd* (figure 21). The basal cell also undergoes a similar division forming two superposed cells (figure 22). Thus, the proembryonal tetrad is linear. The cells *cc* and *cd* by one more transverse division each, form four cells that are arranged in a linear row above the basal cells (figures 23, 24). The two daughter cells of the tier, *cc* are designated as *ce* and *cf* and those of *cd* as *m* and *ci*. In each of the three tiers of cells, *ce*, *cf* and *m*, two vertical divisions take place at right angles to one another resulting in the formation of three superposed tiers of four cells each (figures 25–28).

Periclinal divisions take place in the tier *cf* delimiting the dermatogen, *de*, from a group of inner cells which soon divide transversely resulting in two tiers of cells (figures 29–32). Cells in these inner tiers of *cf* divide transversely and periclinally and the derivatives undergo elongation and differentiation into periblem, *pe* and plerome, *pl* of the hypocotyledonary part of the embryo, *phy* (figures 33–37). The cells of the tier, *ce* contribute to the stem tip, *pvt* and cotyledons, *pco* (figures 30–37). Meanwhile, the derivatives of the cell *m* contribute to all parts of the root, *iec* and *co* (figures 32–37). Cells derived from *ci* and *cb* form a suspensor, *s* (figures 24–37).

Thus, in *Hoppea fastigiata*, cell *ca* of the two-celled proembryo contributes to the development of the entire dicotyledonary embryo and part of the suspensor while the basal cell *cb* contributes to the formation of only a part of the suspensor. Further, in the destination of the cells of the proembryonal tetrad, the embryogeny corresponds to the Physalis II variation of Solanad type (Johansen 1950).

3.6 *Pericarp and seed coat*

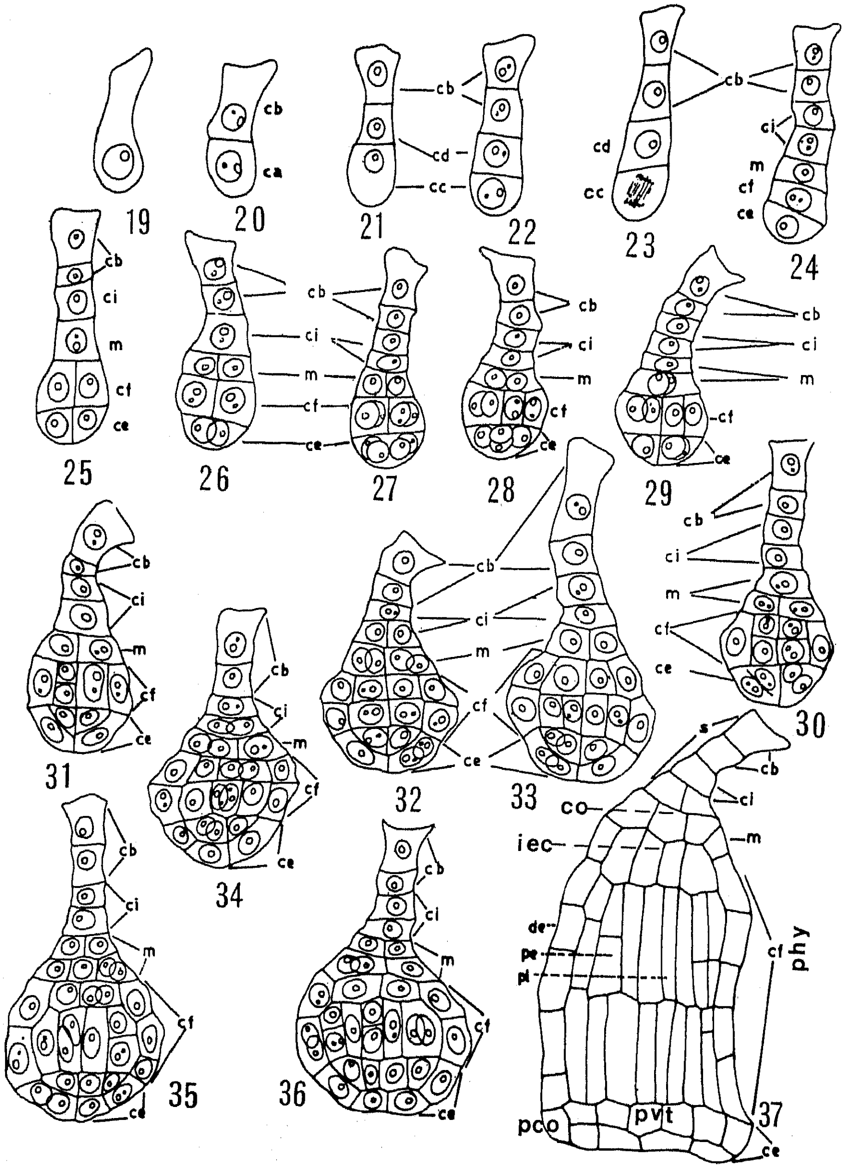
The ovary wall is five to seven cells thick. The outer tangential walls of the ovary epidermis are cutinized. Cytoplasm in all the cells of the ovary wall is highly vacuolate (figure 16). The epidermis of the pericarp is also thickly cutinized and the innermost layer of cells shows tangential elongation. Cells of the hypodermal layers become thick-walled (figure 17).

The epidermis of the integument becomes the seed-coat, the cells of which are thin-walled. The outer tangential walls of the outermost endosperm cells develop lamellar thickenings. This lamellated layer lies directly below the seed coat (figures 15, 18).

4. Discussion

Erythraeinae show considerable variation in their tapetal ontogeny and morphology.

In a relatively simple type of development as recorded in *Enicostemma littorale*



Figures 19–37. **19.** Zygote. **20.** Two-celled proembryo. **21.** Three-celled proembryo. **22.** Proembryonal tetrad. **23–24.** Five to seven-celled proembryos. **25–30.** Proembryo showing the formation of tiers *ce, cf, m, ci.* **31–36.** Advanced stages of proembryos showing the demarcation of histogens. **37.** Heart-shaped embryo (all × 600). (*ca*, apical cell; *cb*, basal cell; *cc*, upper daughter cell of *ca*; *cd*, lower daughter cell of *ca*; *ce*, upper daughter cell of *cc*; *cf*, lower daughter cell of *cc*; *ci*, daughter cell of *cd*; *de*, dermatogen; *m*, daughter cell of *cd*; *pe*, periblem; *pl*, plerome; *co*, root cap; *iec*, initials of root cortex; *pco*, cotyledons; *phy*, hypocotyl; *pvt*, stem tip; *s*, suspensor).

rinivasan 1941), *Canscora diffusa*, *C. decussata* (Maheswari Devi 1962), *Centaurium* *mosissimum* (Vijayaraghavan and Usha Padmanaban 1969) and *Erythraea roxbur-* *ii* (Maheswari Devi and Satyanarayana 1971), the tapetum is derived entirely from e inner parietal layer of the microsporangium and forms a homogenous layer of cells the periphery of the locule.

Tapetal ontogeny of the type described for *Alectra thomsoni* (Vijayaraghavan and atnaparkhi 1973) has been observed in *Hoppea dichotoma*, *Canscora decurrens* ankara Rao 1978; Sankara Rao and Chinnappa 1983b) and *Hoppea fastigiata*. The petum is of dual origin and develops partly from the inner parietal layer and partly om the elements of the connective tissue. The tapetal cells in these species, unlike in *lectra thomsoni*, remain uninucleate and do not accumulate starch.

Of special interest is the tapetal development described in *Canscora pauciflora* ankara Rao and Chinnappa 1983b). Strips of sterile tissue of sporogenous origin and peripheral tapetal layer of parietal origin, together constitute the tapetal complex in is species. Steffen and Landmann (1958) referred to a similar tapetal complex in *entiana cruciata* as Balken or trabeculate tapetum.

The tapetum is glandular in *Chlora perfoliata*, *Erythraea centaurium*, *Cicendia* *liformis* (Guérin 1925), *Enicostemma littorale* (Srinivasan 1941), *Centaurium ramos-* *simum* (Vijayaraghavan and Usha Padmanaban 1969), *Erythraea roxburghii* Maheswari Devi and Satyanarayana 1971), *Hoppea dichotoma* (Sankara Rao 1978) nd *H. fastigiata* in the present study while in *Canscora diffusa*, *C. decussata* Maheswari Devi 1962) and *C. decurrens* (Sankara Rao 1979), it is plasmodial.

Pollen grains are in monads. They are tri- or tetrazonocolporate. Considerable traspecific variation of pollen occurs in *Centaurium erythraea*. A small percentage of ains in *C. erythraea* ssp. *grandiflorum* are syncolpate. Further, in three of the *entaurium* species viz., *C. pulchellum*, *C. exaltatum* and *C. littorale*, pericolporate ains are formed in addition to tricolporate pollen (Sankara Rao and Chinnappa 983a). It should also be noted that the pollen grains of *Canscora*, *Hoppea*, *Sabbatia*, *nicostemma* and *Centaurium* (Sankara Rao 1978, 1979; Sankara Rao and Chinnappa 983b) develop from their germ pores certain cytoplasmic hyaline capitate protrusions. hese protrusions, however, are ephemeral and detached from the grains before thesis.

In the entire group, the ovules are unitegmic. However, the absence of an integument d consequent absence of a micropyle has been reported in the saprophytic *Obolaria* *rginica* (Johow 1885; Holm 1897). According to Oehler (1927), the apparent ategmic ndition of the ovule in this species is due to the fusion of the scanty nucellus with the tegument.

The development of the female gametophyte conforms to the Polygonum type as in e other Erythraeinae. The antipodals in the group show variation in their behaviour. ney are generally weakly-developed uninucleate cells which degenerate at the time of rtilization and belong to the group Ia of the scheme of antipodal classification of olt (1927). In *Hoppea dichotoma* (Arekal 1961), *Centaurium ramosissimum* ijayaraghavan and Usha Padmanaban 1969) and *H. fastigiata*, on the other hand, e antipodal cells which are normally-developed and uninucleate, remain persistent to 2-nucleate stage of endosperm. They conform to group II of the antipodal assification.

Though the nuclear type of endosperm development is a feature common to all ythraeinae, species show variation in the mode of wall formation that eventually

follows. It is centripetal in *Canscora diffusa*, *C. decussata* (Maheswari Devi 1962) and *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969) whereas in *Hoppea dichotoma* (Arekal 1961), *Canscora decurrens*, *C. pauciflora* (Sankara Rao 1979) and *H. fastigiata* it is simultaneous.

Protein granules observed as food reserves in the endosperm cells of *Hoppea fastigiata* have also been recorded in *Erythraea roxburghii* (Maheswari Devi and Satyanarayana 1971), *Canscora decurrens*, *C. pauciflora* (Sankara Rao 1979). In *Canscora diffusa* and *C. decurrens*, however, starch grains in addition to protein granules appear in the endosperm cells (Maheswari Devi 1962).

Judging from the data available, it can be seen that the reproductive morphology of the group is essentially gentianaceous with bisexual hypogynous protandrous flowers, tetrasporangiate anthers and their dicotyledonous wall development, glandular or plasmodial tapetum, simultaneous cytokinesis in the microsporocytes, tricolporate pollen, anatropous unitegmic tenuinucellar ovules lacking integumentary tapetum, Polygonum type of female gamatophyte development, nuclear endosperm, Solanad type of embryogeny and endospermic seeds. They are specialized in so far as they show: (a) a combination of herbaceous habit and saprophytic nutrition as in *Bartonia* and *Obolaria*: (b) halophytic and xerophytic adaptations in *Enicostemma littorale* and *Cicendia filiformis*; (c) aggregation of small flowers into dense inflorescences as in *Faroa* and *Enicostemma*; (d) zygomorphy in the flowers of *Canscora*, *Hoppea* and *Schinziella*; (e) gradual reduction in the male complement of flowers to varying degrees; (f) syngenesious condition of anthers as in *Tapeinostemon*; (g) the three-traced condition of open carpels with parietal placentae (Gopala Krishna and Puri 1962); (h) ategmic ovules as in *Obolaria*; (i) precocious degeneration or delayed division of the upper dyad cell during megasporogenesis in *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969) and *Canscora diffusa* (Maheswari Devi 1962); (j) persistent antipodal cells in *Hoppea* (Sankara Rao 1978 and present study) and *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanaban 1969) and (k) polyembryony in *Erythraea centaurium* (Crété 1949a). These specializations are trends of evolutionary significance in the Erythraeinae.

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A new species of *Brachiaria* Griseb. (Poaceae) from India

G P BASAPPA*

Department of Post-Graduate Studies and Research in Botany, University of Mysore, Manasagangotri, Mysore 570 006, India

* Present address: Department of Botany, D V S College of Arts and Science, Shimoga 577 201, India.

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Abstract. A new species, *Brachiaria munae* Basappa, from South India, is described and illustrated.

Keywords. Poaceae; *Brachiaria munae.*

1. Introduction

While collecting the Indian Brachiarias for biosystematic studies, we have collected a few new taxa of which one is described here. All the herbarium specimens of *Brachiaria* in CAL, MH, BSI, BSJo, BSD, DD, LWG and ASSAM have been examined. However, the present taxon does not resemble any of those and is hence described here as a new species.

2. Results and discussion

2.1 *Brachiaria munae Basappa sp. nov.*

Brachiaria stapfianae Basappa *et* Muniyamma similis spicularum formis, sed differt habitu perfecte prostrato, foliis parvioribus, paniculis racemisque brevibus, partim exsertis, spiculis majoribus, gluma superiore et gluma inferiore 11–13 nervi.

Type: Basappa 3001; India: Tamil Nadu, Madurai Kamaraj University Campus, 20 Dec. 1979 (CAL holotype; MH, BSI, BSJo, MGM isotypes).

B. munae is similar to *B. stapfiana* in spikelet shape, but differs in its completely prostrate habit, smaller leaves, partly exserted short panicles and short racemes, bigger spikelets and the 11–13 nerved lower and upper glumes.

Annual; culms prostrate, profusely branched, rooting at the basal nodes, spreading in rosettes, (20) 26–37 (40) cm long; internodes rarely exceed 5 cm, hirsute; pulvinate nodes and sheaths distinctly and finely ciliate; sheaths rather tumid, striate, softly villous, the terminal (2) 2·6–3·9 (4·2) cm long; ligule a short ciliate rim, 1–1·5 mm long; blades ovate-lanceolate, acute or subacuminate, rounded or subcordate at the base, softly villous, crisped on one of the margins, (3) 4–5·2 (5·5) cm long, (0·7) 0·9–1·2 (1·3) cm wide; panicles short, partly hidden or just exserted from the sheath, (4·5) 5·3–7·7 (8) cm long, (1·8) 2–3 (3·2) cm wide; peduncle below the inflorescence terete and villous, (1·2) 1·3–2·6 (2·8) cm long; inflorescence axis angular, softly villous, terminating with a spikelet; racemes (3) 4–6 (7), secund or subsecund, alternate, the basal 2–3

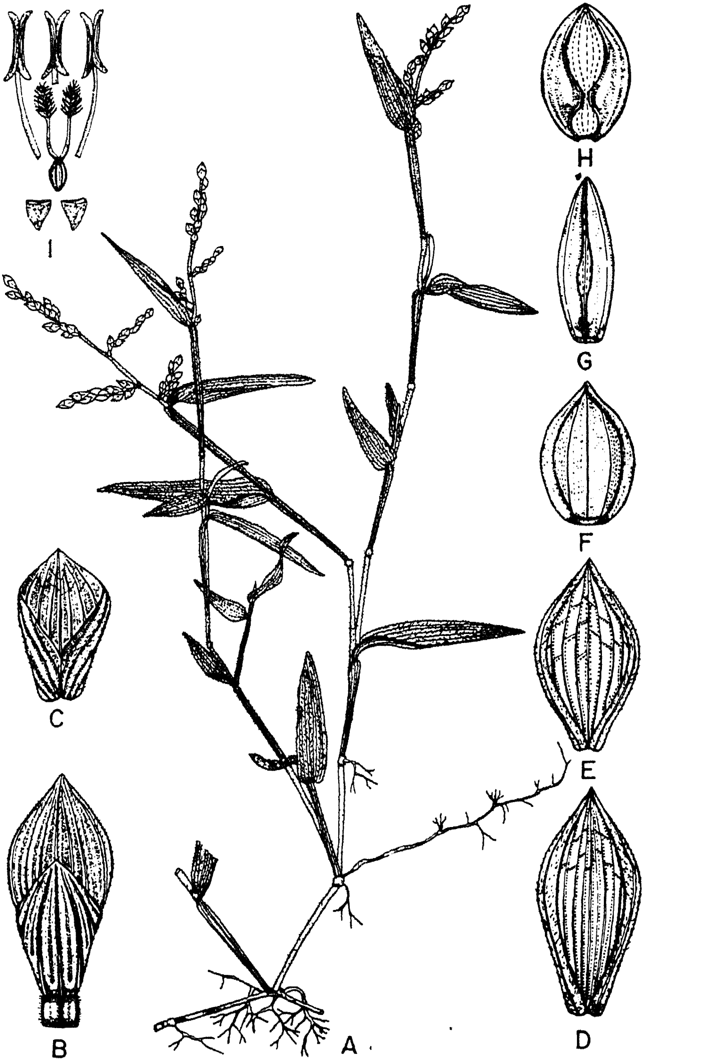

Figure 1. *Brachiaria munae.* **A.** habit; **B.** spikelet; **C.** lower glume; **D.** up

the lower and upper glumes elongated and swollen; lower floret neuter, l
4·06–4·16 (4·2) mm long, similar to the upper glume in texture, 7-nerved, pa
3·82–4 (4·05) mm long, much shorter than the lemma, faintly 3-nerved, tra
upper floret bisexual, broadly elliptic, mucronate, turgid, (2·75) 2·82–3 (
long, (1·75) 1·8–1·92 (1·95) mm wide, lemma and palea coriaceous, finely
polished, the palea (2·35) 2·4–3 (3·05) mm long, lodicules 2; stamens 3, anthe
styles 2, stigmas deeply purple; caryopsis (2·7) 2·75–2·95 (2·98) mm long, (
1·85 (1·9) mm wide, broadly ovate in outline, with rounded ends, embryo 2/3
length, hilum sub-basal; chromosome number $n = 18$.

Ecology: This species typically inhabits sandy soil in open fields and stony sl
elevation below 300 m. Most of the specimens have been collected between N
and February.

Distribution: Brachiaria munae is known only from the type locality. T
distribution in infrequent patches suggests its rarity and endemism.

Etymology: This notably distinct species is named in honour of Dr M Mu
who has greatly contributed to our knowledge of the grasses of this regio

This entity is unique in several constant differences, principally in its prost
without ascending branches, and short panicles partly enclosed within the bo
just exserted, which amply justify its separation from other species. Contra
those of many Brachiarias the racemes are only 4–6 per panicle and the basal
conspicuously the longest comprising seldom more than 10 spikelets. The
number of spikelets produced in any panicle is 30—incidentally it is the lowe
for Indian Brachiarias.

The most distinctive feature of this taxon is its larger spikelets up to 4·75 ×
largest among Indian species. This distinctive character is complemented
nerved glumes, a situation not found in any other Indian species.

For confirmation, this taxon was sent to the Royal Botanic Gardens, Kew
confirmed it as a species of *Brachiaria* not resembling any of the African spec

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herbarium and library facilities. He also thanks Dr N C Majumdar of BSI, H

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Embryology of three species of *Ehretia*

B HANUMANTHA RAO and P S PRAKASA RAO*

Department of Botany, Andhra University, Waltair 530 003, India

* Department of Botany, Nagarjuna University, Nagarjunanagar, (

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Abstract. Embryology of three species of *Ehretia* (*E. ovalifolia*, *E. micr*
described. The anther is tetrasporangiate and the wall corresponds to
type. Reduction divisions in microsporocytes are simultaneous and tetr
decussate. Occasionally, isobilateral tetrads are met with. Polyspory i
grains are usually shed at the two-celled stage. The ovules are anatr
crassinucellar. An endothelium differentiates. Both Polygonum and Al
metophytes coexist. The endosperm is Cellular with four-celled micr
chalazal haustoria. Embryogeny is of the Onagrad type, and suspens

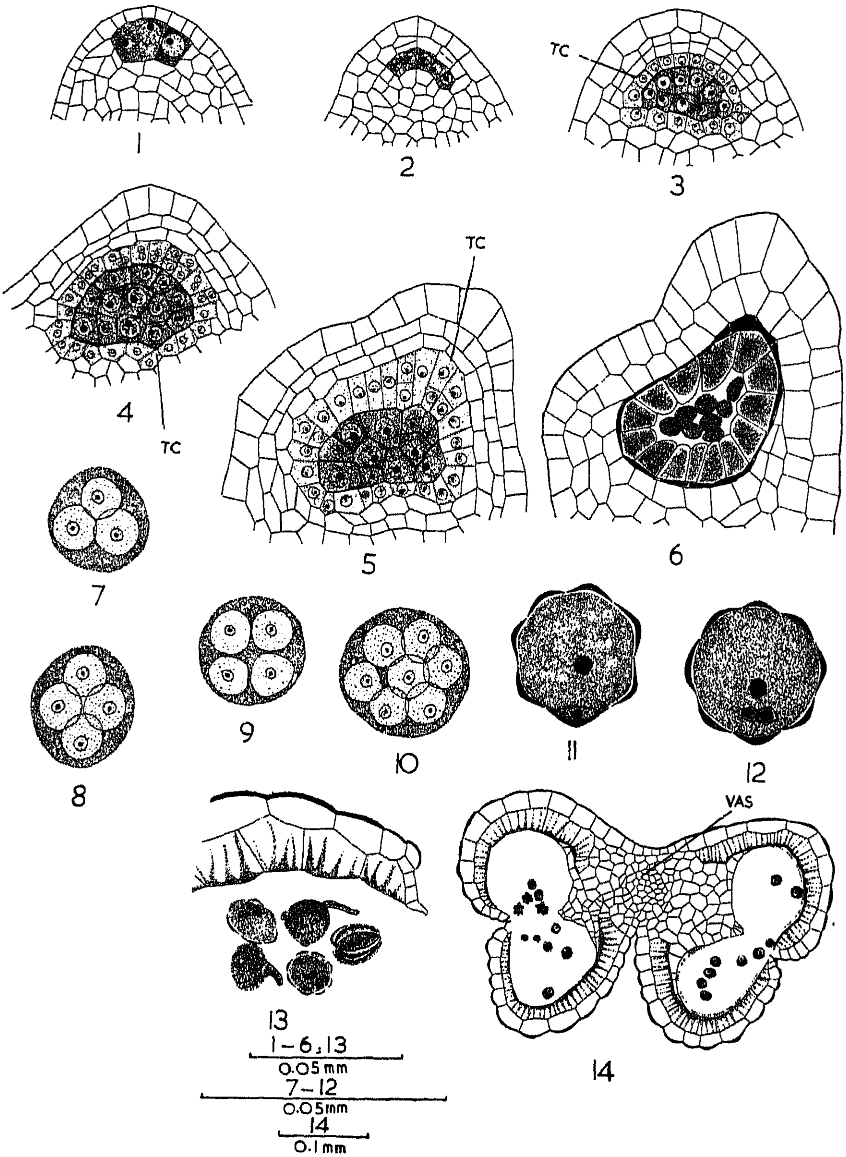
Keywords. Embryology; *Ehretia*; Ehretioideae; Boraginaceae; hausto

1. Introduction

Ehretioideae constitutes the second sub-family of Boraginaceae, wit
species (Engler and Prantl 1897). So far embryological informatic
Ehretia laevis (Johri and Vasil 1956); and *E. acuminata* (Khaleel 197
Ehretia (*E. ovalifolia* and *E. microphylla*) have been studied, and
reinvestigated.

2. Material and methods

Buds, flowers and fruits of *Ehretia ovalifolia* Wt., *E. microphylla* L
Roxb. were fixed in FAA and Carnoy's fluid, and stored in 70% ethan
methods of dehydration and embedding were followed. 6–8 μ th
mounted with Haupt's adhesive, and stained in safranin-fast green



Figures 1–14. Anther, microsporogenesis and male gametophyte. **1–3**; **7–14**. *Ehretia ovalifolia*, **4**. *E. laevis*, **5**, **6**. *E. microphylla*. **1**. TS anther lobe with three-celled archesporium. **2**. Anther lobe with division of primary parietal cells. **3**. Anther lobe with wall layers, tapetum and sporogenous cells. **4**. Anther lobe with partly biseriate tapetum, binucleated tapetal cells and microsporocytes in prophase of meiosis I. **5**. Anther lobe with radially elongated parietal tapetal cells. **6**. Anther lobe with degenerated tapetal and sporogenous cells. **7**, **8**, **9**. Tetrahedral, decussate and isobilateral microspore tetrads respectively. **10**. Polyspory. **11**, **12**. Two-celled and three-celled pollen grains respectively. **13**. Mature anther lobe with germinating pollen grains. **14**. Dehiscing anther. (TC, Tapetal cells; VAS, Vasculature).

time the sporocytes enter meiosis (figures 3, 4). Occasionally, the division of the tapetal nuclei is followed by a transverse wall so that, at places, it becomes biseriate (figures 4, 5). In *E. microphylla* the parietal tapetal cells are radially elongated (figure 5). Meiosis

is synchronous in *E. ovalifolia*, but the synchrony is restricted to one or two locules in *E. microphylla* and *E. laevis*. Rarely, in *E. microphylla* one or two anthers may show signs of degeneration (figure 6). The tetrads are tetrahedral, decussate and isobilateral (figures 7, 8, 9). Polyspory is frequent (figure 10). The pollen grains are two-celled at the shedding stage; occasionally even three-celled (figures 11, 12). Exceptionally, *in situ* germination occurs in 5% of the anthers in *E. ovalifolia* and *E. microphylla* (figure 13). The exine is thick, smooth and ridged (figures 11, 12).

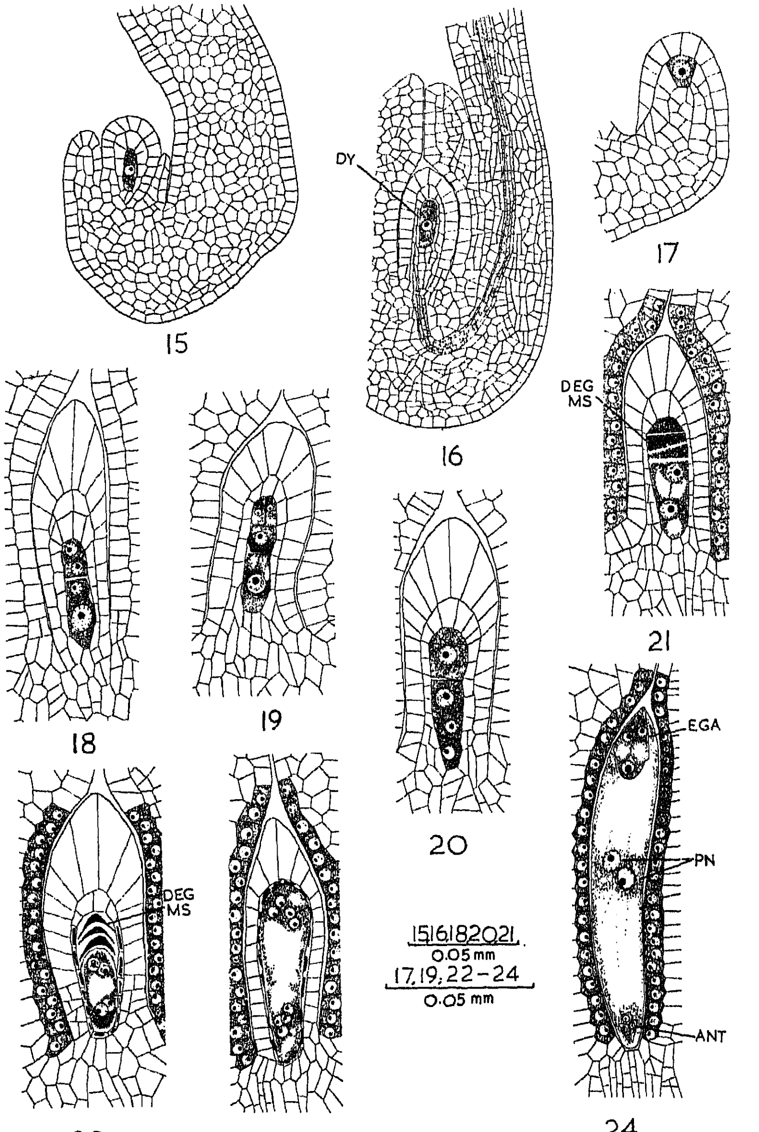
3.2 *Megasporangium, megasporogenesis and megagametophyte*

The ovules are anatropous and unitegmic in *E. ovalifolia*, *E. microphylla* and *E. laevis* (figures 15, 16). An endothelium differentiates from the innermost layer of the integument (figures 24, 31). The vascular tissue arises from the placenta, passes through the funiculus, and terminates at the chalaza (figure 16). A hypostase differentiates in *E. microphylla* (figure 31). An unicelled, hypodermal archesporium becomes discernible prior to the appearance of the integumentary primordium (figure 17). Occasionally, two to three archesporial cells differentiate, but only one of them functions further. The archesporial cell cuts off a parietal cell and a megasporocyte (figure 15). A few-celled parietal layer, develops from the parietal cell. The megasporocyte gives rise to dyads, after the first meiotic division (figure 16). Infrequently, even two dyads are formed in *E. laevis* (figure 25). Linear or *T*-shaped tetrads are formed after second meiotic division (figures 18, 19). In a few preparations of *E. ovalifolia* obliquely oriented linear tetrads have been observed (figure 20). Usually, the chalazal megaspore functions further and the three micropylar megaspores degenerate (figure 21). In 4% of the ovules of *E. microphylla* and *E. laevis* the micropylar megaspore also enlarged (figure 26). Rarely, in *E. laevis* the micropylar megaspore becomes functional, while the others exhibit diverse degrees of development and displacement (figure 27). The functional megaspore enlarges, and its nucleus undergoes three free nuclear divisions resulting in an eight-nucleate megagametophyte (figures 21, 22, 23), of the Polygonum type (Maheshwari 1950). In *E. microphylla* the mature megagametophyte is filled with starch grains (figure 31).

The parietal cells and the adjacent cells become highly compressed and degenerate totally by the time the eight-nucleate megagametophyte is organised (figure 23). The cells of the nucellar epidermis also collapse and degenerate by the mature megagametophyte stage (figures 24, 31). Besides Polygonum type, an occasional tendency to follow Allium pattern has been observed in about 6% ovules in *E. ovalifolia*, 20% in *E. microphylla*, and 20 to 25% in *E. laevis* (figures 28, 29, 30). Fertilization is porogamous (figures 32, 33).

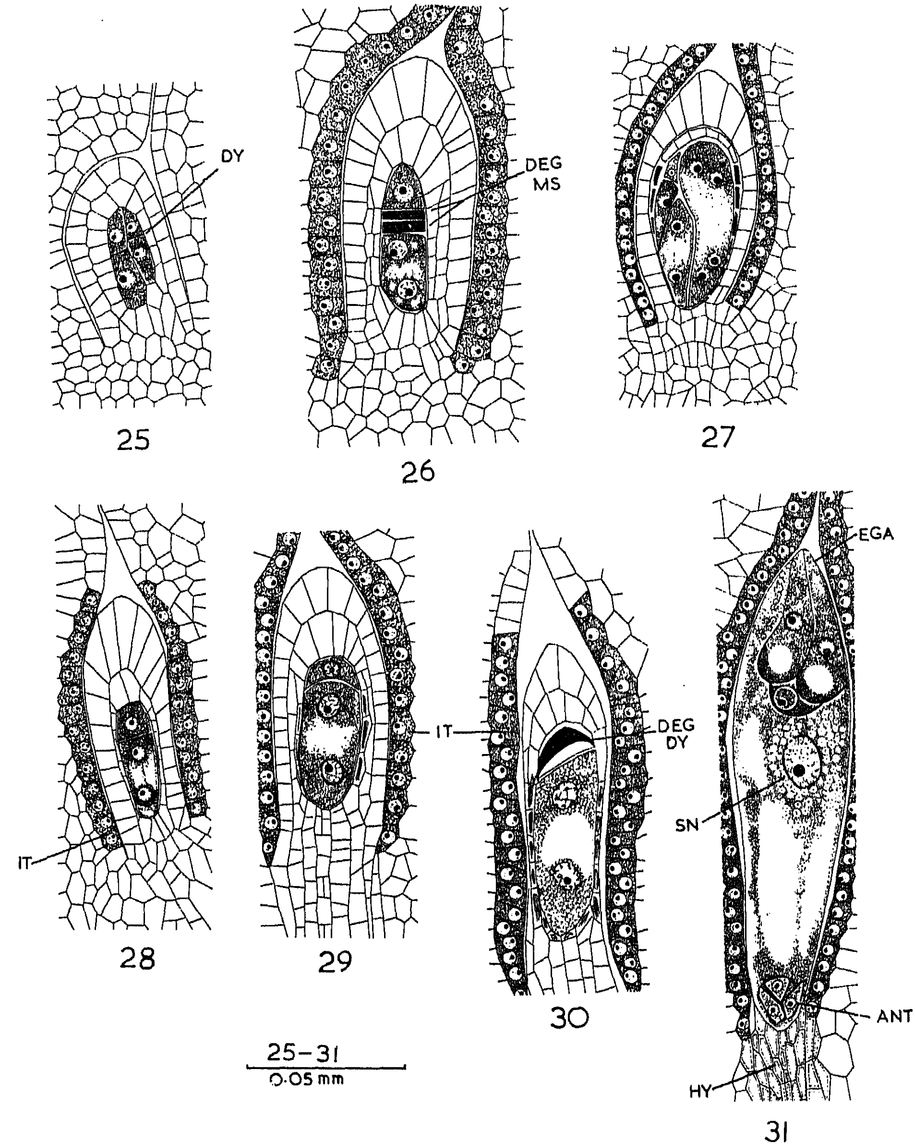
3.3 *Endosperm*

In *Ehretia* species the endosperm is cellular, and corresponds to the Myosotis type of Svensson (1925). The division of the primary endosperm nucleus precedes that of the zygote (figure 34), and is accompanied by a transverse wall (figure 35). Another transverse division in each chamber results in a row of four endosperm cells (figure 36). Repeated transverse and longitudinal divisions, in the two central cells, result in a massive endosperm tissue (figures 37, 38). Concomitant with these divisions, the terminal cell at the micropylar end, after contributing a few more cells to the endosperm tissue, undergoes two longitudinal divisions at right angles to each other resulting in a



Figures 15–24. Ovule, megasporogenesis and female gametophyte in *E. ovalifolia*. **15.** LS unitegmic, anatropous ovule with parietal and megaspore mother cells. **16.** LS ovule with vascular strand and dyad. **17.** LS ovular primordium with single-celled archesporium. **18, 19, 20.** Linear, *T*-shaped and obliquely linear megaspore tetrads respectively. **21.** Two-nucleate embryo sac. **22.** Four-nucleate embryo sac. **23.** Eight-nucleate embryo sac. **24.** Organised embryo sac. (ANT, Antipodals; DEGMS, Degenerating megaspores; DY, Dyad; EGA, Egg apparatus; PN, Polar nuclei).

quartet of cells which organise into the micropylar haustorium (figure 38). The chalazal cell behaves likewise and, ultimately, develops into a chalazal haustorium of four cells (figure 38).



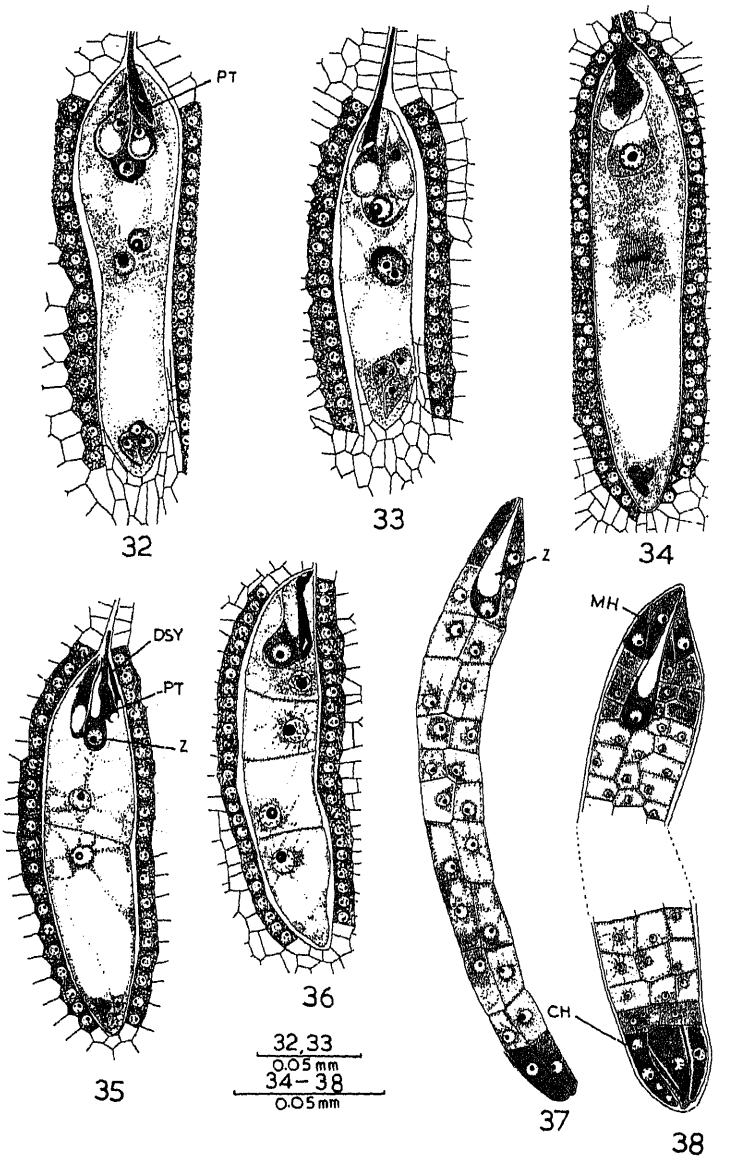
Figures 25–31. Variations in megasporogenesis and female gametophyte. **25–27, 29.** *E. laevis*. **28.** *E. ovalifolia*, **30, 31.** *E. microphylla*. **25.** LS nucellus with parietal cells and dyads. **26.** LS nucellus with enlarged micropylar megaspore; chalazal two-nucleate embryo sac. **27.** Displaced megaspores; developing two-nucleate and four-nucleate embryo sacs. **28, 29, 30.** Degenerating upper dyad cells; functional lower two-nucleate embryo sacs. **31.** Mature embryo sac with starch grains. (ANT, Antipodals; DEGMS, Degenerating megaspores; DEGDY, Degenerating dyad cell; DY, Dyad; EGA, Egg apparatus; H, Hypostase; IT, Integumentary tapetum; SN, Secondary nucleus).

.4 *Embryo*

'he development of embryo is described for *E. microphylla* and *E. laevis*.

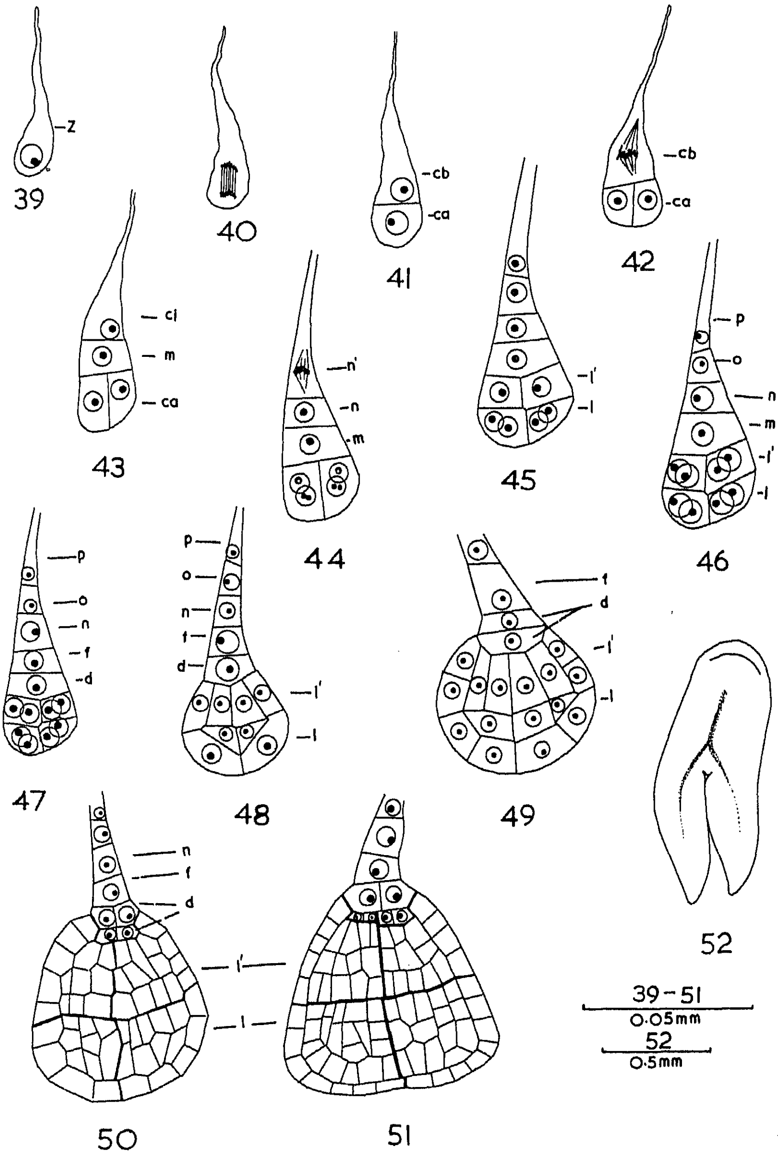
The zygote elongates considerably, and divides transversely resulting in an apical cell

ca and a basal cell *cb* (figures 39, 40, 41). Of these, *ca* divides vertically to form two juxtaposed cells, while *cb* segments transversely giving rise to two superposed cells *m* and *ci* (figures 42, 43). The proembryonal tetrad, thus, conforms to A_2 category of Šouèges (see Crété 1963). Each of the two derivatives of *ca* divides vertically at right



Figures 32–38. Fertilization and development of endosperm. **32, 33, 35, 36.** *E. microphylla.* **34, 37, 38.** *E. ovalifolia.* **32.** Mature embryo sac with pollen tube entered. **33.** Mature embryo sac with remnants of pollen tube and secondary nucleus. **34.** Embryo sac with zygote and dividing primary endosperm nucleus. **35.** Two-celled endosperm. **36.** Four-celled endosperm. **37.** Endosperm with micropylar and chalazal haustoria. **38.** Endosperm with four-celled micropylar and chalazal haustoria. (DSY, Degenerating synergid; CH, Chalazal haustorium; MH, Micropylar haustorium; PT, Pollen tube; Z, Zygote).

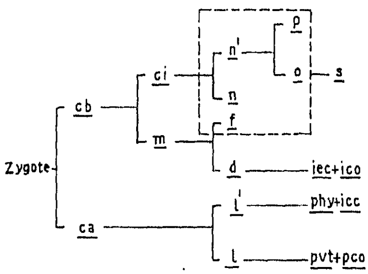
angles to the first wall initiating quadrants (figure 44). By transverse divisions in these cells originates the octant, *l* and *l′* (figures 45, 46). Simultaneously, the cell *ci* segments transversely and engenders two superposed cells *n* and *n′* (figure 44); the latter divides transversely to form *o* and *p* (figure 46). Subsequently the cell *m*, by a transverse division, gives rise to the cells *d* and *f* (figure 47). Periclinal divisions in the cells of the octant (*l* and *l′*) delimit the dermatogen (*de*) from the inner group of cells (figure 48), and the inner cells of the tier *l′* give rise to the periblem (*pe*) and plerome (*pl*) (figure 49). A transverse division takes place in *d*, after the differentiation of dermatogen in the octant,



Figures 39–52. Development of embryo in *E. microphylla*. **39.** Zygote. **40.** Division of the zygote. **41–51.** Stages in the development of the embryo. **52.** Mature embryo. (z, Zygote).

delimiting two superposed cells of which the upper functions as the initial for the root cortex (*iec*), and the lower as initial of root cap (*ico*) (figures 49, 50, 51). Usually these cells produce two plates of four cells each, consequent to two vertical divisions at right angles to each other (figure 51). Later segmentation in these cells results in the organisation of root cortex (*iec*) and root cap (*ico*). In the *l*, which is destined to develop cotyledons and shoot apex, both transverse and longitudinal divisions take place and the embryo eventually passes through globular and heart-shaped stages (figures 50, 51). Further divisions in the tier *l* engenders the cotyledonary region (*pco*) and shoot apex (*pvt*). The tier *l′* contributes to the hypocotyledonary region (*phy*) and the initials of the central cylinder of the stem (*icc*), and the tier *m* and its derivatives to the initials of hypophysis and root cap (*ico*). The cells *f*, *n*, *o* and *p* contribute to the formation of a four-celled suspensor (figure 50). The mature embryo is dicotyledonous with a discernible shoot apex, root and root cap, and the vascular supply to the cotyledons (figure 52).

The embryogeny in *E. microphylla* and *E. laevis* corresponds to the Lythrum variation of the Onagrad type (Johansen 1950), or period I, Megarchetype IV, Series A_2 of the embryonic classification of Šouèges (see Crété 1963), and can be recapitulated by the following schematic representation and embryonic formula:



4. Conclusions

Occurrence of both Polygonum and Allium types of megagametophyte development seems to be common among the species of *Ehretia* (present study; *E. laevis*, Johri and Vasil 1956; and *E. acuminata*, Khaleel 1977) whereas only Polygonum type has been recorded in *Coldenia procumbens* (Venkateswarlu and Atchutaramamurti 1955) and *Rotula aquatica* (Nagaraj and Fathima 1967). Hjelmquist (1964) remarks "....... where 4-celled tetrad and monosporic development occur together with bi-, tri-, or tetrasporic development, the Normal type must be the point of departure". Johri (1963) points out that there is hardly any uncertainty concerning the primitiveness of the Polygonum type of embryo sac. Further he is of the opinion that the Allium and Endymion types can be deduced from Polygonum type by suppression of cell plate formation after Meiosis II and by the elimination of one mitotic division during gametogenesis. Therefore in *Ehretia* also the bisporic condition may be considered as a derived one from the monosporic type.

Among the members of Ehretioideae, the micropylar endosperm haustorium is four-celled, except in *Rotula aquatica* (Nagaraj and Fathima 1967), where it is two-celled. However considerable diversity exists in the organisation of the chalazal haustorium. It is single-celled with a hexaploid nucleus in *Coldenia procumbens* (Venkateswarlu and

Atchutaramamurti 1955); four-celled in the three species of *Ehretia* studied by us, *E. laevis* (Johri and Vasil 1956), and *E. acuminata* (Khaleel 1977); and eight-celled, with circumaxial arrangement in *Rotula aquatica* (Nagaraj and Fathima 1967). Thus, only in the species of *Ehretia* both the haustoria are equally well developed, whereas in the others, no correlation is seen in the organisation of the two haustoria, where one is more developed than the other.

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Variation in the peroxidase isozymes and soluble seed protein patterns of *Vigna radiata* (L.) Wilczek mutants

S M RAO

Department of Botany, Osmania University, Hyderabad 500 007, India

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Abstract. Electrophoretically separated peroxidase isozymes and soluble seed protein profiles of some of the viable mutants of green gram in the *M*4 generation have revealed qualitative and quantitative variations from the parental line. Some of the mutants exhibited increased or decreased soluble protein bands according to the altered genetic architecture. The study of the similarity index of the soluble seed protein profiles indicated varying degrees of homology ranging from 40 to 83% among the parental line and mutants.

Keywords. *Vigna radiata*; isozymes; peroxidases; seed proteins; mutants; similarity index.

1. Introduction

Various workers have employed the protein and isozyme patterns as an additional clue to establish the phylogenetic relationships in higher plants (Fox *et al* 1964; Johnson and Hall 1965; Hart and Bhatia 1967; Siddiq *et al* 1972; Ladizinsky and Adler 1975). Tripathi *et al* (1981) studied soluble protein and isozyme patterns in the anthers of Sorghum with diverse cytoplasmic and nuclear factors to assess the qualitative and quantitative variations and genetic similarities. Farook and Nizam (1978) and Wolff (1980) used the protein patterns in determining the genetic affinity among the mutants. The present study intends to compare between mutants and parental line of *Vigna radiata* (L.) Wilczek for qualitative and quantitative alterations in peroxidase isozymes and soluble seed protein patterns due to mutation process.

2. Material and methods

Eight induced mutants of green gram i.e., (i) early flowering and ripening; (ii) small pod; (iii) white flower color; (iv) anthocyanin type; (v) shiny seed; (vi) highly indeterminate growth; (vii) indeterminate growth and (viii) spreading type were isolated from the various mutagenic treatments (Rao 1982) and selected for the present investigation. All the above mutants were tested for their isolated characters up to M_4 generation and all of them were breeding true.

2.1 *Extraction of peroxidase and soluble seed proteins*

For studying peroxidase isozyme patterns, 500 mg of leaf from ten day old seedlings of each mutant grown in moist filter paper folds at room temperature (25°C) were ground in 0·01 M Tris-HCl buffer (pH 7·5; 1:5 w/v) with a chilled pestle and mortar. The homogenate was centrifuged at 10,000 × g for 15 min at 4°C and 50 μl of the

6

Table 1. Observations on various quantitative characters for isolated mutants.

Plant type	Isolated from	Generation		Days to flowering	Number of pods	Plant height (cm)	Number of branches	Days to maturity	Pod length (cm)	Number of seeds	50-seed weight (gm)	Total seed yield (gm)	% of protein content
Control	—	—	$\overline{X}$	39·70	23·30	38·90	2·80	67·90	7·28	11·47	1·56	7·13	21.67
			S.E.	0·45	1·76	1·47	0·17	0·62	0·12	0·21	0·20	0·45	
			Variance	4·1100	62·2100	43·1900	0·5600	7·6900	0·2797	0·8628	0·0077	4·1108	
Early flowering and ripening	Gamma rays/ M_1 generation	M_3	$\overline{X}$	32·65**	15·10**	24·50**	1·65**	61·30**	6·21**	10·66**	1·45	4·06**	24·99
			S.E.	0·19	0·72	0·85	0·16	0·15	0·08	0·21	0·04	0·22	
			Variance	0·7275	10·3900	14·5500	0·5275	0·6569	0·1559	0·9004	0·0289	0·9552	
		M_4	$\overline{X}$	32·60**	15·65**	26·45**	1·90**	61·35**	6·05**	10·13**	1·58	4.39**	24·89
			S.E.	0·19	1·19	0·65	0·20	0·15	0·08	0·16	0·03	0·43	
			Variance	0·7400	25·0275	8·5475	0·7900	0·6708	0·1448	0·5171	0·0221	3·6860	
Small pod	$E-H/M_1-M_1$†† generation	M_3	$\overline{X}$	37·25**	45·85**	33·60**	2·60	73·80**	4·35**	4·65**	1·60	5·62*	24·15
			S.E.	0·56	3·22	0·82	0·13	0·19	0·09	0·23	0·04	0·40	
			Variance	6·2875	207·0275	13·3400	0·3400	0·7600	0·1712	1·0795	0·0301	3·2417	
		M_4	$\overline{X}$	39·30	49·15**	33·60**	2·55	73·20**	4·51**	5·24**	1·62	5·99	24·18
			S.E.	0·17	4·51	1·03	0·18	0·54	0·10	0·27	0·04	0·50	
			Variance	0·6100	407·2275	21·3400	0·6475	5·7600	0·2047	1·4784	0·0393	4·9678	
White flower	$G-G/M_2-M_1$† generation	M_3	$\overline{X}$	55·30	29·60	24·60**	2·25	77·00**	4·86**	5·90**	1·38	3·56**	21·99
			S.E.	0·75	4·31	1·54	0·25	0·55	0·21	0·58	0·02	0·58	
			Variance	11·5100	371·2400	47·7400	1·2875	6·0000	0·9186	6·6420	0·0105	6·7274	
		M_4	$\overline{X}$	54·20**	20·30	26·85**	2·70	74·95**	5·54**	8·16**	1·36	3·15**	21·86
			S.E.	0·59	1·38	1·14	0·22	0·62	0·13	0·44	0·04	0·20	
			Variance	7·0600	38·0100	25·9725	1·0100	7·7475	0·3386	3·8305	0·0280	0·8287	

Spreading	Gamma rays $+EMS/M_1$ generation	M_4	$\overline{X}$	41·10*	23·10	34·15**	2·55	67·80	6·74**	10·89*	1·37	5·81	
			S.E.	0·50	1·72	0·58	0·11	0·44	0·04	0·14	0·02	0·50	18·95
			Variance	5·0900	59·1900	6·7275	0·2475	3·9600	0·0275	0·3979	0·0108	4·9542	
Indeterminate growth	$G-H/M_2-M_1$†† generation	M_4	$\overline{X}$	48·70**	21·30	54·10**	3·50**	73·00**	6·45**	11·45	1·27	5·57*	
			S.E.	0·69	1·80	0·93	0·16	0·39	0·08	0·25	0·03	0·41	19·16
			Variance	9·6100	65·2100	17·4900	0.500	3·0000	0·1403	1·3115	0·0140	3·4598	
Highly indeterminate growth	$G-E/M_1-M_1$†† generation	M_4	$\overline{X}$	51·30**	16·20**	72·80**	3·40*	78·50**	6·17**	11·16	1·62	5·28*	
			S.E.	2·31	1·53	2·05	0·15	0·30	0·14	0·38	0·02	0·69	18·83
			Variance	53·6100	23·3600	42·1600	0·2400	0·9718	0·2042	1·4784	0·0069	4·2254	
Shiny seed	$G-G/M_1-M_1$† generation	M_4	$\overline{X}$	36·20**	22·60	28·20**	1·80**	66·10**	5·80**	8·08**	1·60	4·58**	
			S.E.	0·28	1·31	0·81	0·09	0·18	0·11	0·27	0·08	0·31	22·49
			Variance	1·6600	34·2400	13·1600	0·1600	0·5676	0·2472	1·4496	0·0353	1·8966	
Anthocyanin pigment type	Gamma rays/ M_1 generation	M_4	$\overline{X}$	39·65	22.83	37·80	2·05*	72·85**	5·98**	9·95**	1·78	5·64*	
			S.E.	0·56	2·16	0·86	0·23	1·02	0·12	0·27	0·04	0·44	22·99
			Variance	6·2275	93·0275	14·9600	1·0475	20·9275	0·2974	1·4955	0·0297	3·9459	

* Significant at 5% level of probability; ** Significant at 1% level of probability; † Recurrent mutagenic treatments of gamma rays (G); †† Sequential mutagenic treatments of Gamma rays (G), EMS (E) and HZ (H); + Combination mutagenic treatment; M_1, M_1-M_1 and M_2-M_1 – First, successive and alternate generation mutagenic treatments.

supernatant liquid was used for electrophoresis. Seed protein was extracted by soaking 500 mg of fine seed powder in 0·5 M Tris-HCl buffer (pH 7·6; 1:20 w/v) for 24 hr. The extract was centrifuged at 12,000 × g for 30 min at 4°C and 60 μl of the supernatant was collected and used for the electrophoresis.

2.2 *Electrophoresis*

Disc electrophoresis was done according to the method of Davies (1964) using 7·5 % polyacrylamide gels in tris-glycine buffer (pH 8·5) at 4°C. A current of 2·5 mA for peroxidase isozymes and 3 mA for soluble seed protein per tube was subjected for 2 hr. After completion of the run, which was indicated by the movement of the tracking dye, the gels were removed and stained.

2.3 *Staining procedure*

For peroxidase, the gels were incubated with saturated benzidine, 30 % ammonium chloride and 0·2 % of H_2O_2 in the ratio of 50:10:2 for 15 min.

The gels were immersed in 7 % acetic acid for 3 min till the bands were deeply stained and preserved in 2 % acetic acid. For seed protein, gels were stained with 2 % Coomassie blue in 12·5 % trichloroacetic acid for 30 min and destained in 7 % acetic acid.

The gels were scanned on 'Shimadzu UV 240' gel scanner at 530 nm and 630 nm for peroxidase and soluble seed protein respectively. The relative migration (*Rm*) of each band and similarity index values were calculated as given in Siddiq *et al* (1972).

3. Results

3.1 *Peroxidase isozymes*

The data on peroxidase band patterns and relative migration (*Rm*) values of eight mutants and control are presented in table 2 and illustrated in figure 1. Peroxidase isozyme pattern was studied due to its known involvement in plant growth and development. The eight mutants included in the present study showed significant differences from the parental line for their isolated characters (table 1). Nevertheless, all of them possessed the similar number of bands i.e., 7, but had their own pattern due to varying band mobilities and intensity. The bands with 0·06, 0·21 and 0·63 *Rm* values were exclusively found in the mutants of white flower, anthocyanin and shiny seed, respectively. Similar band patterns were observed for the control, early flowering, small pod mutant, anthocyanin and shiny seed mutants at *Rm* 0·35 and 0·47. In a comparison, band pattern of white flower color differs entirely from indeterminate growth mutant with the latter one exhibiting closer relation with the parental line. A similar comparison of indeterminate and highly indeterminate growth mutants reveals more or less similar band pattern except at 0·09 *Rm* value. The band at *Rm* 0·53 exhibited highest relative percentage of peroxidase activity in highly indeterminate growth mutant but the same band exhibited very little activity in indeterminate growth mutant. For indeterminate mutant, the band with *Rm* 0·09 exhibited highest peroxidase activity.

Table 2. *Rm* value of peroxidase isoenzyme bands on polyacrylamide gel electrophoresis from ten day old seedlings of isolated mutants.

	Mutant type								
Rm value	Control	Early flowering and ripening	Small pod	White flower	Antho-cyanin	Shiny seed	Highly in-determinate growth	Indetermi-nate growth	Spread-ing
0·06				++					
0·09	+		++					+	
0·11		++		++	++	++	+		+++
0·14			++	++					+++
0·17	++	+				+	+	+	
0·21					+				
0·27	+	+	+			+	+	+	+
0·33				+	+				+
0·35	+	+	+		+	+	+	+	
0·44				+					
0·47	+	+++	+++		+++	+++			++
0·53		+					+++	+	+
0·57	+		+	+	+		+	+	
0·63						+			
0·68		+		+					+
0·72			+		+		+	+	
0·73	+					+			

+++ Very dark; ++ Dark; + Faint.

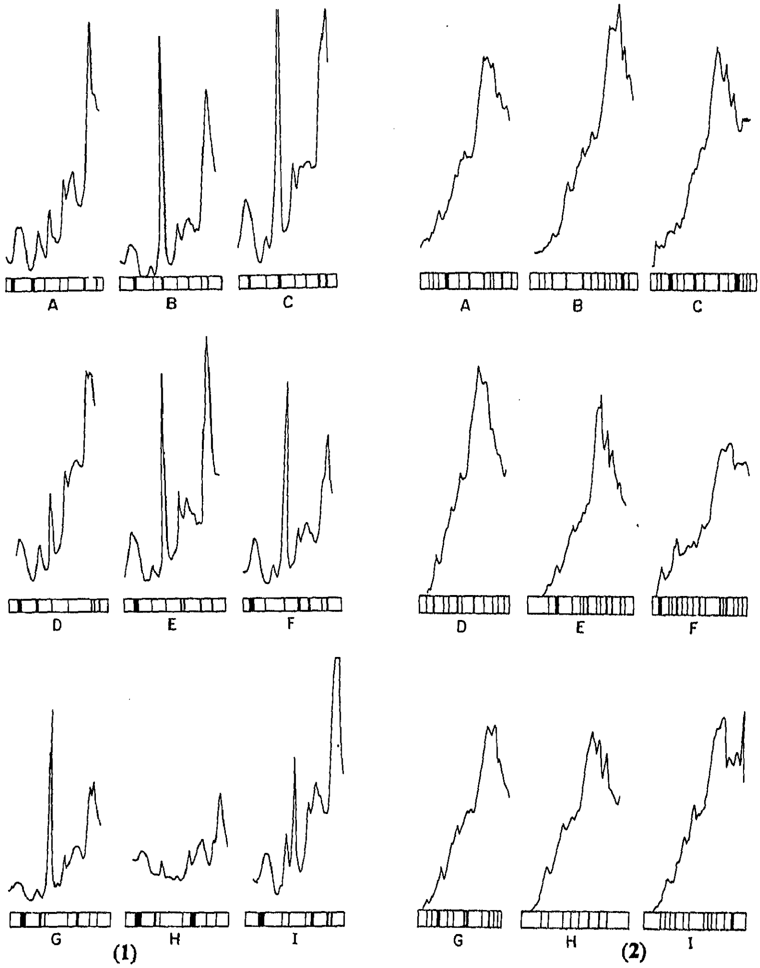
3.2 *Soluble protein patterns*

The soluble protein patterns, relative migration and similarity index values of mutants and the control are presented in tables 3, 4 and illustrated in figure 2.

The results, in general, show that differences exist in the number and intensity of protein bands from the control as well as within the different mutant lines. A total of 11 bands was distinguishable in parental line and a range of 8–15 in the mutant lines (table 3). Among the mutants, indeterminate growth mutant revealed only 8 protein fractions whereas shiny seed mutant a maximum number of 15 bands. In spite of these differences, an overall similarity between the mutants was visible at *Rm* 0·28 as a distinct band visible in control as well as in mutants except in the spreading type. The most exclusive bands were present at *Rm* 0·09 and 0·98 in early flowering and spreading type mutants respectively. In general, the other exclusive protein bands were found at *Rm* 0·44, 0·57 and 0·95 in the mutants of indeterminate growth, anthocyanin, shiny seed and spreading type. The bands at *Rm* 0·21 and 0·17 exhibited high intensity in the early flowering, white flower color and indeterminate growth mutants.

3.3 *Percentage of similarity index*

Percentage of similarities between all possible combinations of mutants and the parental line, based on soluble protein band fractions are summarised in table 4. The homology varied from 58·33 % to 83·33 % between parental line and mutants and 41·17 % to 83·33 % within the mutant lines. The mutants with 11 protein bands showed



Figures 1, 2. Scan of peroxidase isoenzyme and soluble seed protein bands of control and mutants separated by acrylamide gel electrophoresis respectively. **A.** Control. **B.** Early flowering and ripening. **C.** Small pod. **D.** White flower. **E.** Anthocyanin type. **F.** Shiny seed. **G.** Highly indeterminate growth. **H.** Indeterminate growth **I.** Spreading type.

highest homology with the control. However, among all the mutants, indeterminate growth mutant revealed distant similarity with the control (58·33%). Among the mutants, white flower color and shiny seed exhibited highest similarity with the highly indeterminate growth mutant (83·33% and 73·33%). Although early flowering and spreading type of mutants had similar number of bands they showed less homology (41·17%). The small pod mutant showed highest homology with the white flower as well as with the highly indeterminate growth type (71·42%).

4. Discussion

It is presumed that closely related individuals show more similarity in general, than those that are phylogenetically unrelated. According to the definition of species

Table 3. *Rm* value of soluble protein bands on polyacrylamide gel electrophoresis of mutants.

	Mutant type								
Rm value	Control	Early flowering and ripening	Small pod	White flower	Antho-cyanin	Shiny seed	Highly in-determinate growth	Indetermi-nate growth	Spread-ing
0·08		+							
0·14	+	+		+	+				
0·17		+	+		+	+	+	+	
0·21	+	+	+	+	+	+			+
0·28	+	+	+	+	+	+	+	+	
0·30	+			+		+	+		+
0·34		+	+		+	+			
0·36	+	+		+	+		+	+	
0·42			+			+			+
0·44					+			+	
0·47		+			+	+			+
0·50	+						+	+	+
0·52				+	+	+	+		
0·55			+	+					+
0·57					+	+			
0·58	+		+			+	+	+	
0·65		+	+	+		+	+		
0·70	+			+	+	+		+	+
0·73	+	+	+			+	+		+
0·77			+	+	+	+		+	
0·80		+		+			+		
0·84	+		+			+			+
0·87	+	+	+				+		+
0·95			+						+
0·98									+
Total No. of bands	11	12	13	11	12	15	11	8	12

Table 4. Percentage of similarities between control and mutants and in between mutants based on soluble protein component homologies.

Plant type	1	2	3	4	5	6	7	8	9
Control	100·00	64·28	66·66	83·33	64·28	62·50	83·33	58·33	64·28
Early flowering		100·00	56·25	64·28	71·42	58·80	64·28	66·66	41·17
Small pod			100·00	71·42	56·25	64·70	71·42	50·00	56·25
White flower				100·00	64·28	73·33	83·33	72·72	53·33
Anthocyanin type					100·00	55·55	64·28	66·66	50·00
Shiny seed						100·00	73·33	53·33	50·00
Highly indeterminate growth							100·00	58·33	64·28
Indeterminate growth								100·00	53·84
Spreading type									100·00

formation, a new species predominantly arise by mutation process where more or fewer genes are involved. It is assumed that the forms which are closely related differ only in a small number of mutated genes than those which are less closely related (Wolff 1980). It is logical that the similarity between different mutant forms be assessed by studying the proteins or enzymes which are the ultimate products of genes and reflect the genetic constitution of an individual. In the present study, although there was no differences in the peroxidase isozyme band number in control and mutants, the relative mobility values of each band and densitometer scanning analysis indicated a qualitative and quantitative variation in peroxidase activity and subsequently its band pattern (figure 1). As such the occurrence of each isozyme band is controlled by a single gene (Schwartz and Endo 1966) and its intensity by polygenes (McCune 1961), the quantitative and qualitative alterations in the present study could be only due to the pleitropic action of mutated genes as there were no major differences.

In seed proteins, though they were not functionally and structurally undefinable, the gross fractions suggested differences between the mutants and the control. Several number of bands were added or deleted according to the altered genetic constitution of the plant type which could be due to mutations.

The study of similarity index might give an idea about the comparative gene homology between various forms. In the present situation it may be potentially useful in assessing the altered genetic constitution due to the mutation process. This study has indicated different degrees of homology ranging from 40% to 83% between the parental line and mutants. Similarly, the degree of homology also showed variation amongst in-between mutants with equal number of bands being in closer, as well as, in distant relationship. This point itself is an evidence to the fact that, mutagenesis could be useful in inducing variability within the basic genetic constitution and that the mutants obtained in the present study are the products of such induced genetic alterations. In summary, the present study revealed that, mutagenesis could be employed to induce qualitative and quantitative changes in the genetic architecture that were manifested at the biochemical and physiological levels.

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fect of ethephon and amino ethoxy vinyl glycine on heartwood rmation in *Acacia auriculiformis* Cann.

SALMA BAQUI, J J SHAH and G SYAMPRASAD
Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, India

MS received 21 February 1983

Abstract. Ethephon (2-chloroethyl phosphonic acid) and AVG (amino ethoxy vinyl glycine), was administrated in a young healthy tree trunk of *Acacia auriculiformis*. Induction of heartwood occurred as a result of ethephon treatment and it increased with increase in its concentration. AVG treatment showed production of coloured wood only and its formation was more inhibited at a concentration of 500 ppm which indicates that AVG inhibits the action of ethylene produced by even injury to the wood. The sapwood, sapwood-heartwood boundary and heartwood of a normal disc, coloured wood and induced heartwood were histochemically analysed.

Keywords. Ethephon; *Acacia auriculiformis*; amino ethoxy vinyl glycine; heartwood.

Introduction

ıylene is a prime controlling agent in many aspects of plant senescence including the ling of flowers, the ripening of fruits and abscission of leaves (Galston *et al* 1980; :berman 1979). Ethylene is evolved prior to the formation of heartwood (Shain and llis 1973; Nelson 1978). Ethephon is a source of gradually released ethylene gas and :onveniently applied to plant structures (Abeles 1973). The application of ethephon sapwood of *Rhus succedanea* branches by Hillis (1975) resulted in the formation of ɔical heartwood compounds. Heartwood was formed in the young *Azadirachta ica* tree at the site of ethephon application (Shah *et al* 1981). AVG is an inhibitor of ylene action (Baker *et al* 1978). An experiment was therefore designed to ascertain : role of ethylene in the heartwood formation using both an inducer – ethephon and inhibitor–AVG, of ethylene. The present communication deals mainly with the effect these chemicals on production of heartwood in *Acacia auriculiformis*.

Materials and methods

young tree, of *Acacia auriculiformis* Cann, (10 m high) growing in the Botanical rden of the Department was treated with 500 and 1000 ppm of ethephon (Sigma,) and 200 and 500 ppm of AVG (Fluka, Germany) in aqueous solutions at a chest ght. It was injected into holes drilled 30 cm apart. Two replicas for each treatment re also kept along with a control where same amount of mere distilled water was ected. The treatment was given at fortnightly intervals for three times. About three nths after the first treatment the trunk of the tree was analysed for heartwood mation. A longitudinal cut was made in the branch through the respective holes and extent of colour wood and heartwood formation was measured (table 1). Radial

Table 1. Effect of ethephon and AVG on heartwood formation in wood of *Acacia auriculiformis.**

Concentration of ethephon (*e*)/ AVG (*A*) in ppm	Radial extension of the induced colourwood (cm)	induced heartwood (cm)
nil**	10	–
500 (*e*)	nil	12·0
1000 (*e*)	nil	12·5
200 (*A*)	9·0	nil
500 (*A*)	6·0	nil

* The diameter of the control disc was 6·5 cm having 3·5 cm heartwood.
** In case of control 1 ml of distilled water injected in the hole.

longitudinal sections were cut from the treated regions of sapwood and induced heartwood using a sledge microtome.

Histochemical tests were made for starch, lipids, phenols (Baqui *et al* 1979), protein (Fisher 1968), peroxidase, succinate dehydrogenase, acid phosphatase and adenosine triphosphatase (Bancroft 1975). Controls were run with sections boiled in distilled water.

3. Results and discussion

There was a production of coloured wood or heartwood at the site of injury in all the cases. However, the degree of its radial extension varied according to the treatment. (figures 1–3, table 1). The control with distilled water treatment showed formation of coloured wood and heartwood.

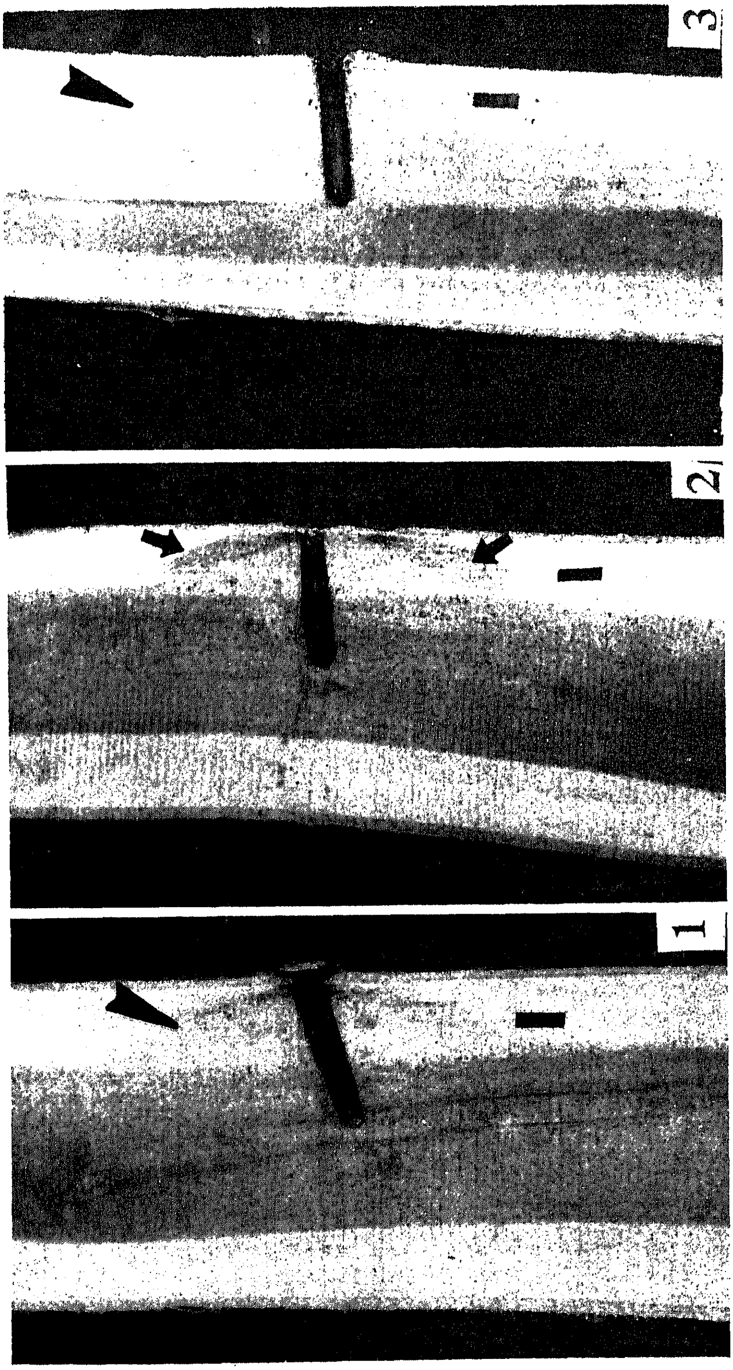
Ethephon treated site showed formation of heartwood. It showed maximum radial extension of the heartwood at a concentration of 1000 ppm (table 1, figure 2).

In contrast to ethephon treatment, there was a formation of coloured wood with the treatment of AVG, but the heartwood formation was negligible. This inhibitory effect on heartwood formation was more at higher concentration of AVG (500 ppm) (table 1, figure 3).

Histochemical observations of various regions such as sapwood, sapwood-heartwood boundary, and heartwood at a normal untreated site, coloured wood and induced heartwood at the treated site are recorded in table 2. The coloured wood showed traces of succinate dehydrogenase, acid phosphatase, ATPase and intense peroxidase activities and decrease of starch grains and deposition of abundant phenolics in ray parenchyma cells and vessels (table 2, figures 4, 5, 7).

The induced heartwood was devoid of any of the enzyme activity and it showed total absence of starch grains and presence of abundant phenolics (figure 6).

The sapwood of a normal branch showed traces of succinate dehydrogenase, acid phosphatase, ATPase and intense peroxidase activities, but moderate activity of



Figures 1–3. Vertical section of a trunk of *A. auriculiformis* showing induced heartwood (arrow) and coloured wood (arrow head) as a result of ethephon and AVG treatments respectively. (1) Control (2) Ethephon, 1000 ppm and (3) AVG, 500 ppm. Bars represent 1 cm.

Table 2. Histochemical changes associated with wood treated with AVG and ethephon.

	Activity/concentration in the tissue				
	Control*			Treated	
Parameter	Sapwood	Sapwood/ Heartwood boundary	Heart- wood	Coloured wood	Heart- wood
Starch	+++	+	−	+	−
Lipids	+	++	−	++	−
Proteins	+	++	−	++	−
Phenolics	−	++	+++	++	+++
Peroxidase	+++	+++	−	+++	−
SDH	+	++	−	+	−
ATPase	+	+	−	+	−
APase	+	+	−	+	−

* The normal disc of the trunk from untreated region. The activity or localization is represented as +++, intense; ++, moderate; +, in traces and −, absent or undetectable.

succinate dehydrogenase and ATPase at the sapwood-heartwood boundary. This data suggests that some of the metabolic parameters studied in the coloured wood have similarity to the sapwood-heartwood boundary of the normal branch (table 2).

The reduced amount of starch in the coloured wood indicates its degradation and possible involvement for the formation of phenolics in induced heartwood. Ethylene causes increase in amylase activity (Kramer and Kozlowski 1979), which brings about degradation of starch. More intensity of lipid localization associated with disappearance of starch grains from the same cells suggest possible conversion of starch into lipids. The breakdown sugars of starch are possibly shunted into biosynthesis of lipids in this region.

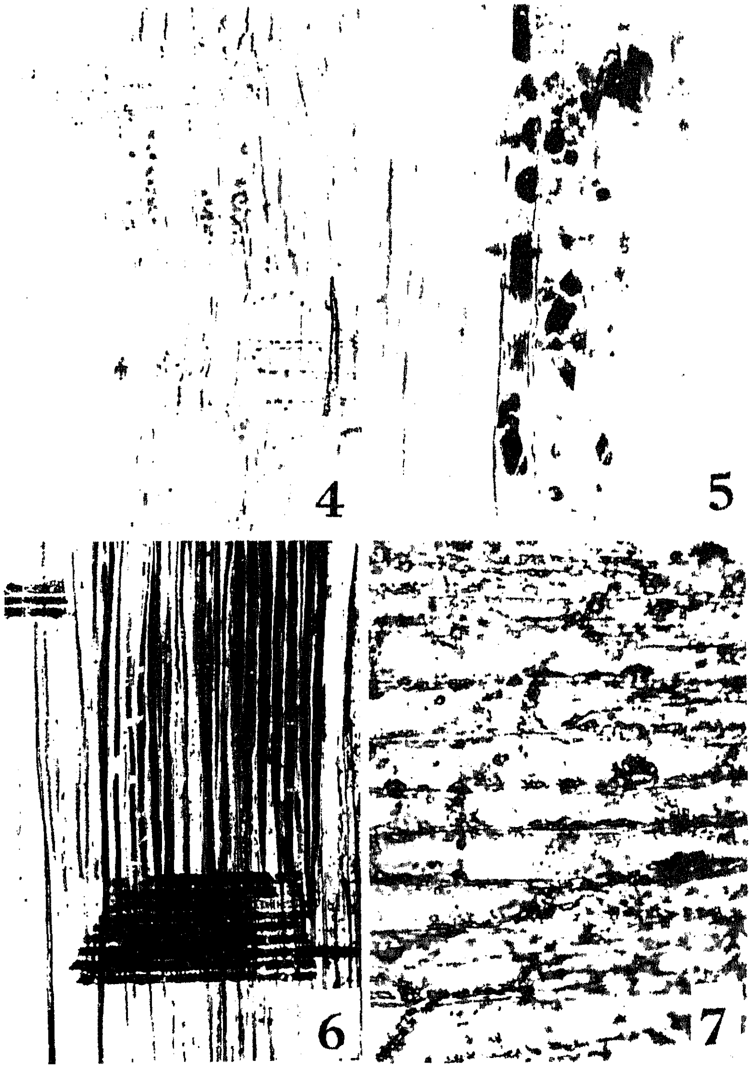
Ethephon treated wood of *Azadirachta indica* (Shah *et al* 1981) showed similar results. Increase in lipid content in coloured wood and its absence in heartwood suggests its possible involvement in the production of heartwood extractives in ethephon treated *A. auriculiformis*. The production of heartwood phenolics from starch is perhaps through the mediation of lipid.

Moderate protein staining is due to its high metabolic state prior to senescence and death.

Increase in phenolics at the sapwood-heartwood boundary, coloured wood and induced heartwood is in agreement with previous report that ethylene plays a key role in the formation of heartwood extractives (Shigo and Hillis 1973). Akhundo *et al* (1979) found similar increase in polyphenol contents in ethrel treated apples. Elevated peroxidase activity at sapwood-heartwood boundary and coloured wood indicates ethephon effect. Ethylene stimulates peroxidase activity in many herbaceous plant species (Abeles 1973).

Some APase activity in coloured wood and sapwood-heartwood boundary in *A. auriculiformis* indicates its involvement in the autolysis and death of the parenchyma cells. High respiration prior to senescence is indicated by SDH activity in this region. Similar results were obtained in ethephon treated *Azadirachta indica* (Shah *et al* 1981).

Traces of ATPase activity at the coloured wood and sapwood-heartwood boundary



Figures 4–7. Radial longitudinal sections of *A. auriculiformis*. **4.** Very few starch grains in the axial parenchyma cells of coloured wood (× 124). **5.** Phenolics in the axial parenchyma cells of coloured wood (× 2140). **6.** Phenolics in ray parenchyma and fibers in induced heartwood (× 124). **7.** Ray parenchyma cells showing intense peroxidase activity in coloured wood (× 2140).

suggests that the process of heartwood formation is energy demanding.

Apparently, mechanical pressure or wounding can provide the intercellular environment that leads to a rapid increase in ethylene synthesis (Osborne 1978). Formation of "artificial heartwood" by boring through the sapwood has been observed in the Beech tree trunk and many conifers and broad leaved trees (Higuchi *et al* 1967). Mere injury into the sapwood induces heartwood formation due to wound ethylene production. AVG is an inhibitor of ethylene action (Baker *et al* 1978). This chemical reduced the heartwood formation considerably, and the production of

coloured wood was also very meagre at 500 ppm concentration. These results confirm the role of etheylene in the production of heartwood.

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Primary production and consumption in the deciduous forest ecosystem of Bandipur in South India*

S NARENDRA PRASAD and H C SHARATCHANDRA†

Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India

† Present address: Department of Zoology, School of Life Sciences, North Eastern Hill University, Shillong 793 014, India

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Abstract. The net annual aerial production of the herb layer in the dry deciduous forest at Bandipur in South India was estimated. *Axis axis* feeds on 38 species of plants; spending 67–95 % of their time on grazing alone. The seasonal trophic activity of *Axis axis* shows that they spend more time feeding in summer than in monsoon period and this was related to the availability of forage. Grass constitutes the major food item in their diet and the foraging range of a herd varies from 22·56 hectares.

The total food consumption of *Axis axis* and *Lepus nigricollis* together was estimated. These two herbivores together consume about 30 % of the net aerial primary production of the herb layer. The *Axis axis* population in addition, consume fruits in the dry period from January to May.

Keywords. Primary production; herb layer; *Axis axis*; herbivore consumption; deciduous forest.

1. Introduction

The Bandipur Tiger Reserve (11° 39′ N lat., 76° 37′ E long.), abutting the Nilgiri hills and adjoining Mudumalai, Wynad and Nagarhole wildlife sanctuaries is one of the largest contiguous forest tracts in South India. It is spread over an area of 689 km^2 and harbours some of the richest populations of wildlife (Nair *et al* 1978). These include elephants (*Elephas maximus*), chitals (*Axis axis*), sambars(*Cervus unicolor*), black-naped hares (*Lepus nigricollis*), wild pigs (*Sus scrofa*), grey langurs (*Presbytis entellus*), and giant squirrels (*Ratufa indica*). We have been engaged in a long term programme of ecological monitoring of this sanctuary complex with special emphasis on the tourism zone of Bandipur Tiger Reserve. The population dynamics of larger mammals was investigated by Sharatchandra and Gadgil (1976) and Johnsingh (1982). The programme aims at understanding the energy flow through the vegetation-chital-elephant-wild dog-panther-tiger food web. As a part of this programme, we report here the results of studies on (a) primary production of the herb layer, (b) the food and feeding behaviour of chital, and (c) food consumption by chital and blacknaped hare populations. These constitute the first careful and quantitative estimates of production and herbivory from South India (Singh and Joshi 1979).

* Contribution No. 4 from the Centre for Ecological Sciences, Indian Institute of Science.

2. Materials and methods

2.1 *The locality*

The investigations were carried out in the tourism zone of the Bandipur Tiger Reserve Forest (figure 1). It has an undulating terrain with an average elevation of 1000 m. The mean annual temperature is 24°C and the annual precipitation around 1000 mm, with a range of 800–2000 mm. The rainfall is restricted from April to November (figure 2).

2.2 *Geology*

This region is a part of the Archean complex. The underlying rock is granite and granite gneisses with intrusions of basic rocks, such as doleritedyk. In addition, schist beds and charnockite rocks may occur. The terrain includes undulating to rolling pediments consisting of (i) hilly areas, (ii) undulating pediments, and (iii) valley fills.

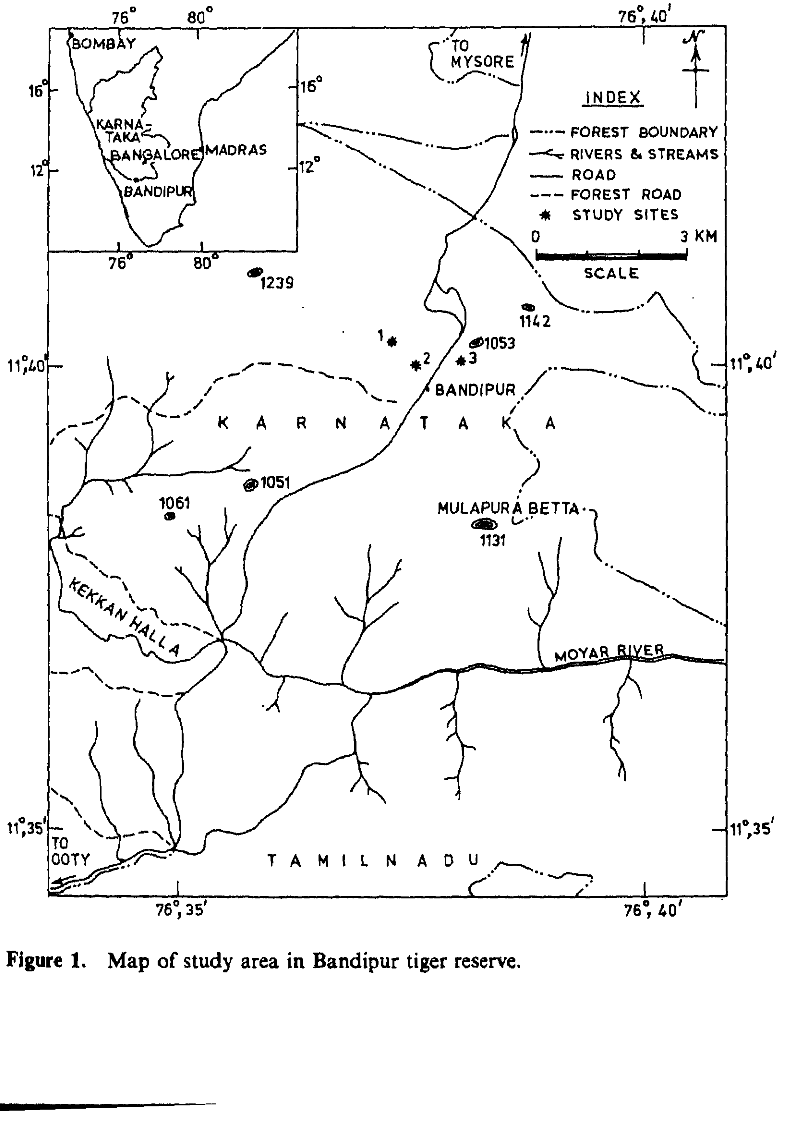

Figure 1. Map of study area in Bandipur tiger reserve.

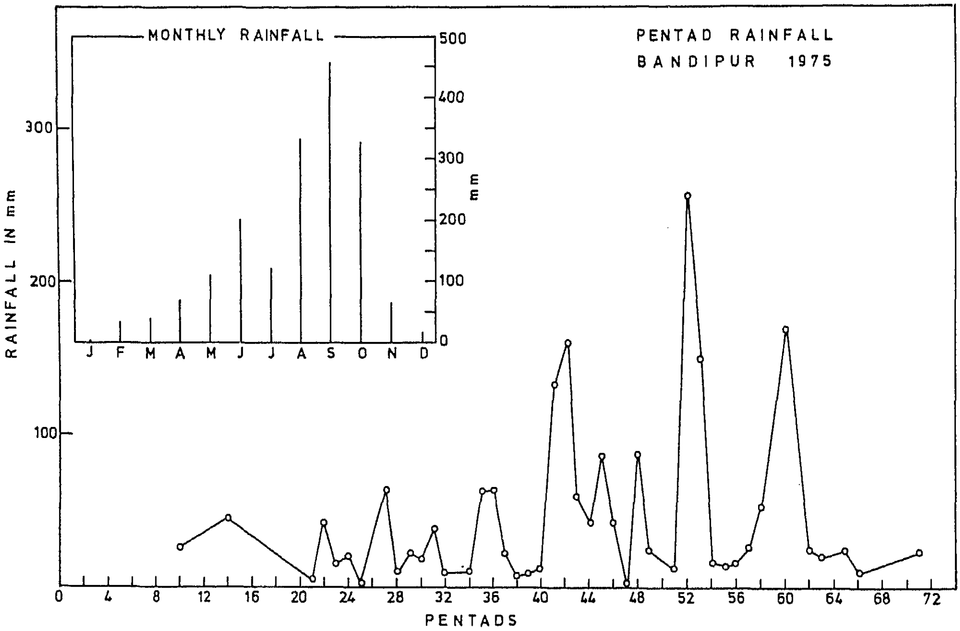

Figure 2. Monthly and pentad rainfall at Bandipur in 1975.

The soils of the hilly area are gravelly, skeletal and rest on weathered parent material or base rock. The soils of the undulating pediments are moderate to deep-gravelly to sandy, resting on weathered plant material. The valley fills consist of deep to very deep soil, which are sandy-loam to sandy-clayey-loam in texture. The soils of the three regions are red or brown in colour.

2.3 *The vegetation*

The vegetation is of dry deciduous type with trees of 10–15 m height and is characterized by an open canopy with considerable undergrowth (figure 3). The major tree species include *Anogeissus latifolia, Terminalia alata, Emblica officinalis, Butea monosperma, Tectona grandis, Xeromphis spinosa,* and *Lagerstroemia parviflora*. The extensive undergrowth consists of *Lantana camara, Dendrocalamus strictus, Givotia rottleriformis, Toddalia asiatica, Argyreia cuneata, Chromalaena odorata, Asparagus racemosus* and *Cryptolepis buchnanii*. The dominant genera of grasses are *Heteropogon, Themeda* and *Cymbopogon* and of herbs *Lepidagathis, Oxalis* and *Curculigo*.

2.4 *Sampling sites*

Three sampling sites were chosen to represent different levels of canopy cover, depth of soil and intensity of grazing (figure 3). The tree densities per hectare at sites 1, 2, 3, were 360, 100 and 150 respectively; a tree being defined as one with a girth of 5 cm or more at a height of 132 cm from the ground level. The shrub growth was extensive on site 1 and minimal on sites 2 and 3. Chital (*Axis axis*) populations were sighted more often on sites

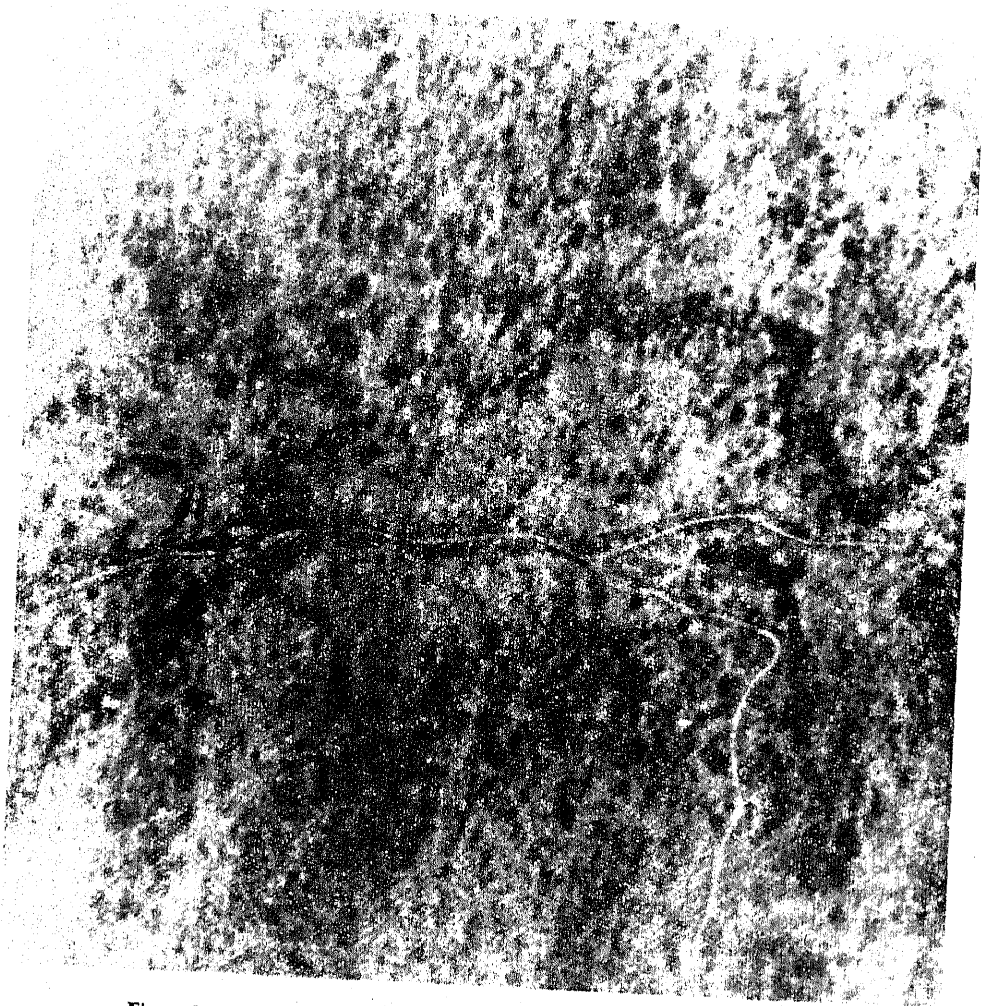

Figure 3. An aerial view of the deciduous forest ecosystem at Bandipur.

2 and 3 and less on site 1. The black-naped hare (*Lepus nigricollis*) was observed occasionally on all sites. The sites 1, 2 and 3 were increasingly closer to the Bandipur lodge area in that order and the soil depth decreased from 1 to 3. The second and third sites experienced heavy grazing by domestic cattle (*Bos indicus*) numbering about 50.

2.4a *Sampling method*: Investigations were carried out for eight months from June 1975 to January 1976. The frequency of observation was once a month; except on sites 2 and 3 data could not be collected in September 1975 due to reasons beyond our control. At each observation, 25 randomly selected quadrats of 50 × 50 cm were located in a hectare. From each of these quadrats, the herb layer was clipped to the ground level (Milner and Hughes 1968). The harvested herbaceous vegetation was sorted out into different species and oven-dried at 80°C to constant weight. The harvested vegetation included live as well as recent dead tissues but not the litter. The net aerial production

was then computed by summing the peak standing crop of current live plus recent dead material of each species (Singh *et al* 1975).

2.5 *Food and feeding behaviour of chital* (*Axis axis*)

Detailed observations were kept on a single herd of chital which spent the night in the lodge area and foraged during the day in the forest to the north-west of the lodge (Bolgudda hillock). These observations included the time activity budget (Sharatchandra and Gadgil 1980) using the focal animal method with randomly related subjects. Over 6500 behavioural sequences were recorded in this manner between 10 October 1975 and 31 September 1976. The observations were restricted to day time from 0530 to 1900 hr with the help of field glasses on the food items, usually identified to the species level except in the case of grasses. The movement pattern of this herd was determined by following it for an entire day once a month from November 1975 to October 1976. A total census of the chital in the 153·2 ha study area was carried out once a month moving slowly along a fixed path, criss-crossing the entire study area.

In addition, general observations were recorded on the usage of habitat and movement pattern of the herds which helped in the design of sampling the faecal matter output of chital.

2.6 *Food consumption by chital* (*Axis axis*) *and black-naped hare* (*Lepus nigricollis*)

The food consumption estimates are based on measuring the total production of faecal matter by the two populations in the study area (Golley 1967; Golley and Buechner 1969; Petrusewicz and Macfadyen 1970). The faecal matter produced was in the form of pellet groups on which four types of measurements were carried out.

2.6a *Rate of production of pellet group by chital and hare*: The sampling design was a stratified random method. Ninety-nine circular plots, each with an area of 30 m^2 were chosen. The lodge and Bolgudda areas in which the habitat usage was intensive, were sampled with a frequency of 0·3 %, while the plantation habitat which was less often used was sampled at an intensity of 0·1 % (figure 4). The sampling was carried out from 1 November 1975 to 31 October 1976. At the beginning, all the plots were cleared of existing pellet groups. Observations were maintained on the accumulations of pellet groups at an interval of ten days. For each of the pellet group we recorded (a) the volume in ml and (b) diameter in mm of an average pellet. The number of seeds of plants voided by chital was also recorded.

2.6b *Standing crop of pellet groups of chital*: To serve as a crosscheck (§2.6a) data were collected on the total number of chital pellet groups over an area of 10 ha once in February 1976 and again in August 1978. For this purpose, a series of 50 transects, each of 20 m length and 10 m width were laid.

2.6c *Mean life span of pellet group of chital*: To correct for disintegration of pellets over the ten-day period of sampling, and to compare the two methods in §2·6a, b an estimate of the mean life span of a pellet group was required. The data for this were based on daily recordings of new pellet groups produced over the last 24 hr and older pellet groups remaining intact. These observations were maintained for 20 sampling

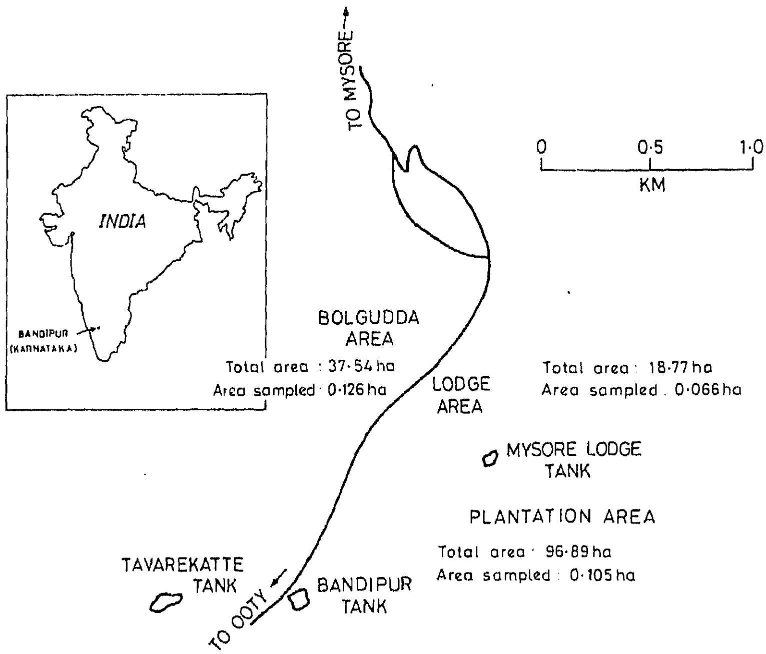

Figure 4. The three pellet production study sites in Bandipur.

plots of 30 m^2, each distributed randomly over the study area. The plots were cleared initially and the pellet groups were then permitted to accumulate for 120 days during July to October 1977. The mean life span based on this data was estimated by developing a stochastic model described in detail by Gore *et al* (in preparation).

The mean life span of a pellet group of chital was 15 days. This implies that the standing crop of pellets is expected to be 15 times the daily production rate. A certain fraction of pellets would also disintegrate over the 10-day interval. Since the mean residence time of pellet groups in this interval would be 5·5 days, a fraction of 0·22 of the pellets would disintegrate. Therefore, the actual pellet production would be 1·25 times that recorded in the field in the 10-day interval.

2.6d *Conversion of pellet group numbers into pellet oven dry weights*: In order to convert the pellet group numbers into oven dry weight, a linear regression was developed. The oven dry weights (y) at 25 randomly chosen chital pellet group were regressed on the volume (x) of these pellet groups. The additional information on the average diameter of pellet was also used in a separate regression relationship. The linear regression was of the following relationship

$$y = -0{\cdot}262 + 0{\cdot}729x,$$

the regression being significant at $P < 0{\cdot}01$ ($F = 113{\cdot}39$ for $[1, 24]$ d. f). The addition of diameter as second independent variable in the regression did not significantly improve the relationship. This information was, therefore, not used.

2.6e *Silica content of herbage and of pellets*: Our estimates of food consumption

were based on the use of silica (SiO_2) as a tracer. Since silica is known to be totally indigestible, its intake in food should equal its output in the pellets. The following relationship can, therefore, be used to relate pellet production to food consumption

$$\begin{array}{c}\text{Total pellet production}\\ \text{(in dry weight)}\end{array} \times \frac{\text{Proportion of silica in dry pellets}}{\text{Proportion of silica in dry forage.}}$$

To use this relationship we estimated silica content in about 100 g of randomly mixed pellets of chital and black-naped hare, and in 100 g of a random sample of herbage fed upon by chital. These were estimated for all the twelve months. Silica content in hare pellets, however, was determined once.

2.7 *Sources of errors in food consumption estimates*

The three sources of errors are (i) no separate regression relationship for estimating the dry weights of hare pellets was obtained. (ii) The mean life span of hare pellet groups was not computed. (iii) The silica content of hare pellet was not determined at monthly intervals. Thus the estimates of hare forage consumption are not as good as those for chital.

The other possible source of error lies in the difficulty in determining the silica content of an average sample of herbage consumed by chital and hare. We collected whatever the chital seemed to eat, mixed and drew a sample from it. This may not be totally adequate. However, the silica content of forage does not vary over a wide range and hence the error may not be appreciable. Further, chital do ingest the same soil as well; and this may be difficult to correct for.

3. Results

3.1 *Herb layer production estimates*

The total monthly standing crop is given in table 1. Our estimates of the total annual net aerial herb layer production in g/m^2 are 305·6, 299·4 and 222 for sites 1, 2 and 3 respectively.

The most productive group of plants for all the 3 sites was grasses, followed by sedges in site 1 and dicot herb in sites 2 and 3. The productive groups in the decreasing order were grasses, sedges, monocot herb, dicot tree and herb, monocot and dicot shrub, dicot and monocot climbers, (table 2). The production everywhere was dominated by *Heteropogon contortus* followed by *Themeda sps* on sites 1 and 2 and *Lepidagathis cristata* on site 3.

Table 2 gives the peak biomass for the nine species groups. There was a tendency towards a postponement to attain peak standing crop with a decrease in the net production.

3.2 *Population dispersion of chital*

Table 3 gives the monthly sightings of males, females and young chital in the study area. Between October and February, relatively small number of individuals occurred. Further, a greater proportion of males seemed to leave the study area during this period.

Table 1. Total monthly standing biomass in three sites (g/m^2).

Month	Site 1	Site 2	Site 3
June	103·14 ±21·14	–	–
July	104·22 ±23·37	102·88 ±22·26	89·73 ±17·73
August	228·40 ±52·29	109·25 ±24·48	154·52 ±32·28
September	93·03 ±62·43	–	–
October	292·53 ±75·27	215·36 ±58·33	164·83 ±32·92
November	244·71 ±75·30	272·60 ±73·80	147·17 ±31·00
December	187·96 ±51·69	221·80 ±61·53	165·18 ±39·60

Table 2. The peak biomass of nine species groups and the month in which it was attained (g/m^2).

Groups	Site 1	Site 2	Site 3
Grasses	253·12 Oct.	274·16 Nov.	133·58 Dec.
Sedges	23·94 Aug.	8·74 Aug.	14·93 July
Monocot herb	13·13 Aug.	26·63 July.	14·07 Aug.
Dicot herb	8·39 Oct·	9·11 Oct·	30·50 Oct·
Monocot climber	0·03 July	0·25 Dec.	–
Dicot climber	0·03 Oct.	–	–
Dicot shrub	1·55 Oct.	0·82 Nov.	1·51 Nov.
Monocot shrub	1·72 Aug	–	–
Dicot tree	8·61 Oct.	2·16 Dec.	2·24 Aug.

Table 3. The number of males, females and young in the study area of 153·2 ha, and the foraging range of one particular herd in hectares of this study area in different months of the year.

Months	Males	Females	Young	Total	Foraging range (ha)
January	21	42	8	71	43·8
February	78	97	23	198	40·6
March	119	139	42	300	40·6
April	101	131	48	280	43·8
May	107	141	49	297	56·3
June	120	136	71	327	21·9
July	113	146	82	341	21·9
August	120	130	80	330	31·2
September	90	126	71	281	24·9
October	26	93	40	159	25·0
November	13	56	19	88	–
December	28	64	20	112	43·8

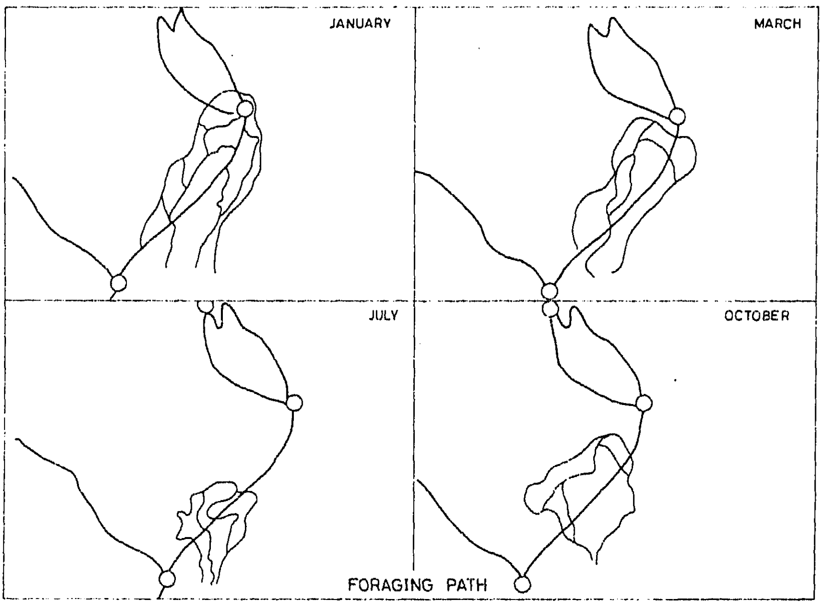

Figure 5. Daily foraging paths of a focal herd of chital.

Table 3 also gives the total foraging area of a focal herd. This area fluctuated considerably from week to week in the premonsoon months from March to May. This was notably less in the monsoon months from June to December, and much larger in the remaining period. Figure 5 shows the foraging paths followed by the focal herd in a single day in four different months of the year. The shift of foraging area from month to month and was the same being minimal in July and maximum in January.

3.3 *Seasonal trophic activity of chital*

Figure 6 gives the percentage time allocated for feeding and ruminating in winter, summer, monsoon and autumn seasons. The percentage time devoted to feeding decreases in that order and correspondingly the ruminating time increases from winter to autumn.

3.4 *Food preferences of chital*

Table 4 lists all the species observed to be eaten by chital. Of all the items, chital seems to favour grasses. In fact, 88·4 % of the feeding activity was devoted to grazing grass. It has fluctuated between 95 % in the rainy season and 67·68 % in the dry months of January and February. During these two months, about 25 % of feeding time was spent on browsing leaves of *Lantana camara* and *Toddalia asiatica*. Over the year, chital devoted 6·1 and 3·8 % of their feeding time on ingesting *Lantana camara* and *Toddalia asiatica* respectively.

3.5 *Pellet production by chital*

Of the three sampling locations, the lodge area had the maximum pellet production of 9·72 kg/ha/day followed by Bolgudda area with 1·02 kg/ha/day and the eastern plantation area with 0·67 kg/ha/day. This reflects the fact that a large number of chital spent the night in the lodge area and preferred the Bolgudda area to the plantation area for grazing during the day-time.

Figure 7 gives the chital pellet production for the year. It fluctuated between a minimum of 1·45 kg/ha/day in October and 2·30 kg/ha/day in June. The production was especially high between May and August and low during March, April, September, October and November. The rise in pellet production was perhaps due to some fresh grass growth following fires. The low level of pellet production in March-April

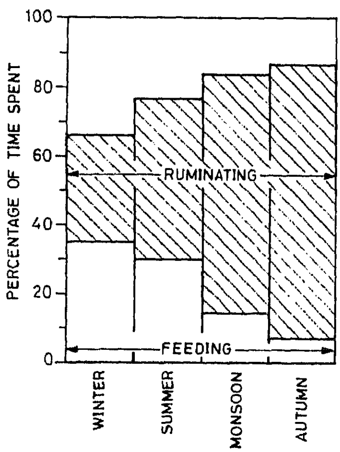

Figure 6. Percentage of day-time spent in feeding and ruminating in different seasons.

Table 4. Plant species consumed by chital.

Species	Parts eaten: Leaves	Flowers	Fruits	Species	Parts eaten: Leaves	Flowers	Fruits
Acacia arabica	+	–	–	*Lantana camara*	+	+	+
Acacia chundra	+	–	–	*Lepidagathis cristata*	+	–	–
Anogeissus latifolia	+	–	–	*Lagestroemia parviflora*	+	–	–
Asparagus racemosus	+	+	–	*Mangifera indica*	+	–	–
Bambusa arundinacea	+	–	–	*Melia dubia*	–	–	+
Bridelia retusa	+	–	–	*Pterocarpus marsupium*	+	–	–
Butea monosperma	+	–	–	*Samanea saman*	+	+	+
Calotropis gigantea	+	–	–	*Tectona grandis*	+	–	–
Capparis sp	+	–	–	*Terminalia tomentosa*	+	–	–
Cassia fistula	+	–	–	*T. bellerica*	–	+	+
Cordia myxa	–	–	+	*T. chebula*	+	–	+
Cymbopogon sp	+	–	–	*Themeda sp*	+	–	–
Dendrocalamus strictus	+	–	–	*Toddalia asiatica*	+	–	–
Desmodium sp	+	–	–	*Vitex altissima*	+	–	–
Emblica officinalis	+	–	+	*Xeromphis spinosa*	+	–	+
Gewotia rottleriformis	–	–	+	*Zizyphus xylopyrus*	–	–	+
Gmelina arborea	–	–	+	*Z. jujuba*	–	–	+
Gymnosporia montana	+	–	–	*Z. oenoplia*	+	–	+
Heteropogon contortus	+	–	–				

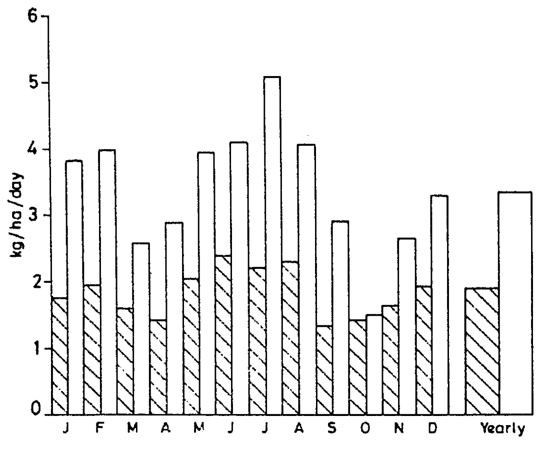

Figure 7. Monthly pellet production by chital and the estimated herbage consumption.

appeared to correlate with the low availability of forage in these months (Sharatchandra and Gadgil 1976).

An independently estimated pellet production, based on two observations in February 1976 and in August 1978 (§2.6b) came to 6000 and 4358 pellet groups respectively. Given that the mean weight of a pellet group was 48·33 g, and the mean life span was 15 days, we get estimates of pellet production of 1·93 kg/ha/day and

1·40 kg/ha/day which is of the same order of magnitude obtained by the first method (§2.6a).

3.6 *Silica content in the forage and in pellets*

Table 5 gives the silica content in forage and in pellets for the 12 months. The percentage fluctuated from 2·54 in January to 5·85 in August. Correspondingly, the silica percentage in pellets fluctuated from 4·5 in November to 10·39 in August. The low value of 3·47 in October was probably an experimental error. The annual average in the forage and pellets was 4·076% and 7·258% respectively. Thus the silica concentration ratio in the pellet was 1·78. These ratios are proportional to the digestibility of herbage. This ratio was at its peak in July corresponding to fresh grass growth and at its lowest during October to December and March, again corresponding to the low digestibility of herbage.

3.7 *Herbage and fruit consumption by chital populations*

Figure 7 gives the estimated herbage consumption by chital. These values range from 2·48 in December to 5·16 kg/ha/day in July, with an annual average of 3·3 kg/ha/day. The second method, described in §§2·6b and 3·5 gives 2·96 kg/ha/day; an estimate of similar order of magnitude. There were about 230 chital in the study area. Hence the consumption by chital by the two methods works out to be 2·2 kg/chital/day and 1·97 kg/chital/day respectively.

The fruit consumption estimates of chital are given in table 6. The estimate is 0·053 kg/ha/day, lower than herbage consumption by a factor of 60. However, as figure 8 shows, very little fruit consumption occurred beyond the January–May period. For this period alone, the consumption rate was 0·128 kg/ha/day, still lower by a factor of

Table 5. Monthly production of faecal matter, silica content in faecal matter and in food, and estimated herbage consumption by chital.

Months	Silica percentage in dry: Fecal matter	Silica percentage in dry: Herbage	Silica concentration ratio
January	5·488	2·537	2·16
February	7·777	3·760	2·07
March	7·536	4·688	1·61
April	9·257	4·680	1·98
May	9·500	4·950	1·92
June	8·580	4·890	1·75
July	9·610	4·200	2·30
August	10·388	5·850	1·78
September	6·238	3·575	1·74
October	3·465	3·400	1·02
November	4·505	2·810	1·60
December	4·700	3·580	1·33
Yearly	7·528	4·076	1·78

Table 6. Total consumption of kg dry weight of the pulp from fruits of various species by the chital population.

Species	Dry weight of pulp per fruit gm	Total weight kg consumed
Terminalia bellerica	3·17	1509·1
Gmelina arborea	3·3	554·8
Terminalia chebula	3	532·8
Melia dubia	1·85	238·5
Emblica officianalis	2·62	42
Zizyphus xylopyrus	1 (approx.)	37·7
Xeromphis spinosa	5 (approx.)	10·6
Zizyphus jujuba	1 (approx.)	4·5
Others	2 (approx.)	8·2

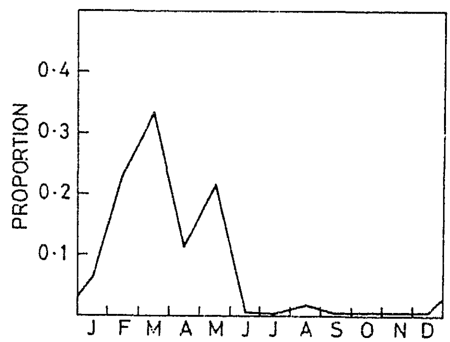

Figure 8. Monthwise proportion of fruit consumption by chital.

25, compared to the herbage consumption. For a population of 230, the consumption was 0·035 kg/ha/day for the entire year or 0·085 kg/chital/day for the January–May period.

3.8 *Herbage consumption by hare*

The estimated daily herbage consumption in the three areas came to 0·8 kg/ha at the plantation, 0·43 kg/ha at the lodge area and 0·15 kg/ha at Bolgudda area. The total herbage consumption over the entire area was 18% that of chital, namely 0·50 kg/ha/day. There were wide fluctuations in the consumption patterns compared to

chital. They fluctuate between the maximum and minimum by a factor of 13, while the maximum and minimum consumption in chital fluctuated only by a factor of 2.

4. Discussion

The method of summation of peak standing biomass of live plus recent dead material provides amongst the highest estimates, if the sampling is weekly or biweekly (Singh *et al* 1975). In the present study, because of the low frequency of sampling, the production was probably an underestimate. Site 3 with its heavy grazing by domestic cattle gave a low value.

The accuracy of our estimate on food consumption of chital seems to be the right order of magnitude as Eisenberg and Lockhart (1972) indicate a population average weight of 45 kg for chital; suggesting a food requirement of around 1·65 kg/chital/day. Our estimates are somewhat high, probably because of the inaccuracies in the estimates of chital population.

The weight of the fruit consumed by chital can be estimated at 0·1 kg/ha/day as the ratio of seed weight to pulp is 0·95. Unlike the herb layer production, we did not directly measure the fruit production. However, an attempt can be made at estimating it. The leaf litterfall in Indian deciduous forests averages 6 tonnes/ha/year or 16·4 kg/ha/day (Garg and Vyas 1975). Data from other tropical forests show the fruit fall to be 5 % of the leaf litter fall (Malaisse *et al* 1975; Wiegert 1970). This suggests a total fruit fall of 0·8 kg/ha/day. Chital, however, consumes, fruit from a restricted range of species (table 4). The proportion of these species in the vegetation was determined through a total enumeration of one hectare plot and it came to 0·35. The production of fruit consumed by chital may, therefore, be estimated as 0·28 kg/ha/day. The chital population, then, must consume about 35 % of the pulpy fruit produced. A further fraction will be consumed by other larger mammals as well as frugivorous birds. Thus the fruit crop is as avidly consumed as herbage by various herbivores.

The extent of forage consumption by domestic cattle is probably of the same order of magnitude as that of chital. The forage requirements of about 50 cattle in the area would be around 100 g/m^2. If this be so, the total annual primary production by the herb layer may be estimated as 433–528 g/m^2. This may be compared with values of 37–1750 g/m^2 for 22 tropical savanna and related communities and 82 to 3396 g/m^2 for tropical grasslands (Bourliere and Hadley 1970; Singh and Joshi 1979). Our values fall towards the lower part of this range. This is to be expected since considerable production must be occurring in the shrub and tree component of the forest ecosystem. Murphy (1975) suggests that the expected value of the total (i.e. above as well as below ground) net primary production of deciduous forest of Bandipur is 1630 g/m^2/year. Since atleast 30 % of the total herb layer production must be below ground production, the total annual herb layer production at Bandipur is likely to range from 620–730 g/m^2. Thus the herb layer production would constitute about 40 % of the expected total production of this deciduous forest ecosystem.

The herbivore consumption including that of domestic cattle and to some extent by elephant, may be around 50 % of the net aerial primary production of the herb layer. Chital and hare themselves consume around 30 % of the production. Thus the deciduous forest ecosystem of Bandipur seems to be one of the most heavily grazed ecosystem in the world comparable to East African grasslands where the mammalian

rbivores have been estimated to consume betwèen 18 and 60% of production /iegert and Evans 1967; Sinclair 1975).

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Two new bladderworts from South India

PETER TAYLOR
The Herbarium, Royal Botanic Gardens, Kew Surrey TW9 3AE, England

MS received 8 March 1984

Abstract. Two new bladderworts *Utricularia cecilii* sp. nov. and *Utricularia lazulina* sp. nov. from South India are described.

Keywords. *Utricularia cecilii*; *Utricularia lazulina*; bladderworts; new species.

1. ***Utricularia cecilii*** P. Taylor sp. nov. affinis *U. uliginosae* Vahl sed corolla grandissima labello superiore multolatiore, calycis margine integro, seminis obovoideis testae cellulis elongatis differt.

Type: India, Karnataka, South Kanara, Kulshekar, *Taylor* 18020 (K holo, BLAT, BSI, CAL, JCB, L, US iso).
Pollen not examined by Huynh, K. L., Étude de la morphologie du pollen du genre *Utricularia* L. *Pollen et spores* 10:11–55 (1968) [4-colporate. 28 × 31 μ *Taylor* 18020 (K)].

Small annual terrestrial. Rhizoids moderately numerous, capillary, up to 1 cm long, tapering, from 0·25–0·05 mm thick, bearing several short, minutely papillose branches. Stolons few, capillary, sparsely branched, a few cm long, *ca* 0·2 mm thick; internodes 2–5 mm long. Leaves few, solitary at each stolon node, petiolate, lamina narrowly obovate, *ca* 1 mm wide, with apex rounded, 3-nerved; total length up to 1 cm. Traps rather few on the leaves and on the stolon internodes, globose, stalked, subdimorphic, those on the leaves with a shorter stalk of equal thickness throughout, those elsewhere with the stalk distally ± thickened, 0·5–0·75 mm long, the mouth basal with 2 dorsal, subulate, glandular appendages of varying length. Inflorescence erect, solitary, 5–17 cm long; peduncle angular, narrowly winged, glabrous, 0·5–0·8 mm thick. Scales few, similar to the bracts. Bracts basifixed, broadly ovate-deltoid with apex acute, *ca* 1·5 mm long, 3-nerved; bracteoles subulate, shorter than the bract. Flowers 1–5, the inflorescence axis elongate; pedicels erect at anthesis, ± spreading in fruit, filiform, narrowly winged, 2–5 mm long. Calyx lobes slightly unequal; upper lobe broadly ovate, 3–5 mm long with apex acute, lower lobe slightly shorter with apex minutely bidentate. Corolla 1–1·5 cm long, deep violet with a white, violet-nerved patch at the base of the lower lip; upper lip constricted below the middle, the superior part broadly obovate with apex rounded, the inferior part much narrower, quadrate; lower lip limb galeate, approximately circular with apex rounded and with a very prominent, obscurely 3-ridged swelling at the base; palate with a pronounced marginal rim, distally pubescent; spur subulate, straight, with apex very acute, about as long as and held at an obtuse angle to the lower lip. Filaments straight, *ca* 1·5 mm long; anther thecae distinct. Ovary ovoid, dorsiventrally compressed; style distinct and relatively broad; stigma lower lip semicircular, upper lip much smaller. Capsule ovoid, *ca* 3 mm long, the wall uniformly

membranous, dehiscing by a single longitudinal ventral slit. Seeds obovoid, *ca* 0·25 mm long with a terminal hilum, reticulate, the testa thin with cells elongate-polygonal and with $\pm$ distinct intercellular spaces (figures 1 and 2).

Distribution. India: Karnataka, South Kanara, vicinity of Mangalore! Known only from the type and *Hohenacker* 71 (W).

Habitat: Shallow wet soil over laterite and "*ad rivulos*" (Hohenacker). Flowering in August and September.

U. cecilii P. Taylor is named after Father Cecil Saldanha, S. J. who showed the author this plant and many others in Karnataka in August 1981 and who has made a very large contribution to the knowledge of the flora of the region. It superficially resembles *U.*

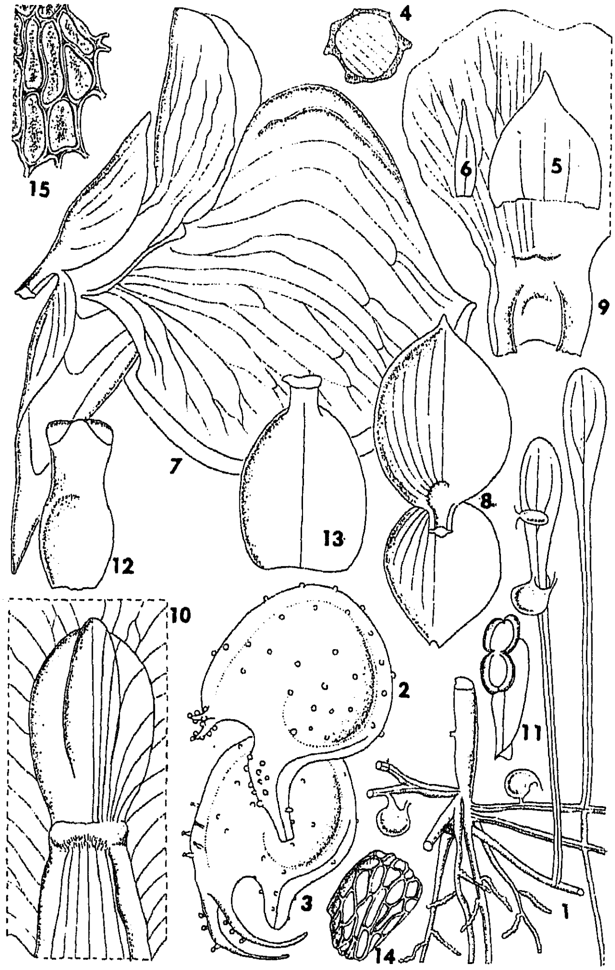

Figure 1. *Utricularia cecilii* P. Taylor **1.** base of plant (× 7·5), **2.** trap from stolon (× 36), **3.** trap from leaf (× 36), **4.** TS of peduncle (× 18), **5.** bract (× 18), **6.** bracteole (× 18), **7.** flower, lateral view (× 7·5), **8.** calyx (× 7·5), **9.** corolla, upper lip (× 7·5), **10.** corolla palate (× 7·5), **11.** stamen (× 12), **12.** pistil (× 12), **13.** capsule (× 7·5), **14.** seed (× 67.5), **15.** testa (× 180).

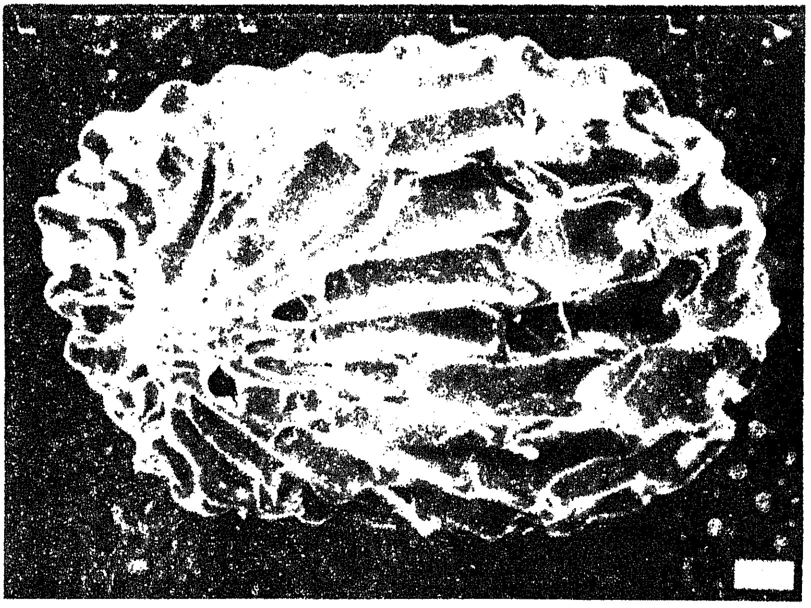

Figure 2. *Utricularia cecilii* P. Taylor. SEM image of seed

reticulata (the Hohenacker collection was so named) with which, in the type locality, it grows. However, the many differences in the vegetative parts, the calyx, capsule and especially the seeds make confusion with it inexcusable. It is possibly more nearly allied with *U. uliginosa*, which has a somewhat similar capsule, but has a very much smaller corolla and minutely denticulate margins to the fruiting calyx lobes and somewhat different seeds.

2. *Utricularia lazulina* P. Taylor *sp. nov.* affinis *U. albocaeruleae* Dalz. sed corolla minore labello superiore multo angustiore, testa minute verrucosa spatiis intercellulis carens distinguenda.

Type: India, Karnataka, South Kanara, Kulshekar, *Taylor* 18021 (K holo, BLAT, BSI, CAL, JCB, L, US iso).
Pollen not examined by Huynh, K. L. *ibid* [3–4 colporate, 25 × 30 μ, *Taylor* 18021 (K)].

Small annual terrestrial. Rhizoids few, capillary, a few mm long, tapering, from *ca* 0·2–0·05 mm thick, bearing several short, minutely papillose branches. Stolons few, capillary, sparsely branched, a few cm long, *ca* 0·2 mm thick; internodes 2–4 mm long. Leaves rather few, solitary at the peduncle base and at each stolon node, petiolate; lamina obovate, mostly *ca* 1 mm wide, with apex rounded, 3-nerved; total length 3–8 mm. Traps rather few on the leaves and on the stolon internodes and sometimes on the rhizoids, globose, stalked, subdimorphic, those on the leaves with a shorter stalk of equal thickness throughout, those elsewhere smaller and with the stalk distally ± thickened, 0·5–0·75 mm long, the mouth basal with 2 dorsal, subulate, glandular appendages of varying length. Inflorescence erect, solitary, 5–10 cm long; peduncle

filiform, angular, narrowly winged, glabrous, 0·6–0·8 mm thick. Scales few, similar to the bracts. Bracts basifixed, ovate-deltoid, with apex acuminate, *ca* 1 mm long, nerveless; bracteoles subulate, about as long as the bract. Flowers 1–3, the inflorescence axis short; pedicels erect at anthesis, spreading or reflexed in fruit, filiform, narrowly winged, 2–5 mm long. Calyx lobes slightly unequal; upper lobe ovate, 2·5–4 mm long with apex acute; lower lobe slightly longer with apex minutely bidentate. Corolla 0·8–1 cm long, clear bright blue with a white, blue-nerved patch at the base of the lower lip and sometimes some white on the upper lip; upper lip very slightly constricted below the middle, the superior part narrowly oblong with apex rounded, the inferior part broadly ovate-deltoid; lower lip galeate, approximately circular with apex truncate and

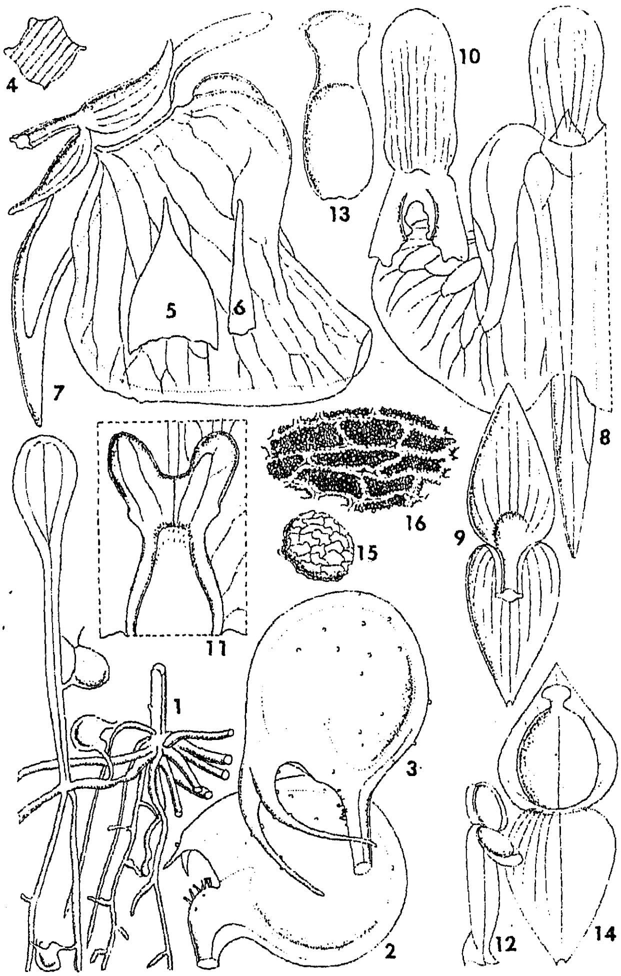

Figure 3. *Utricularia lazulina* P. Taylor **1.** base of plant (× 7·5), **2.** trap from stolon (× 36), **3.** trap from leaf petiole (×36), **4.** TS of peduncle (× 18), **5.** bract (× 18), **6.** bracteole (× 18), **7.** flower, lateral view (× 7·5), **8.** flower, front view (× 7·5), **9.** calyx (× 9), **10.** corolla, upper lip (× 7·5), **11.** corolla palate (× 7·5), **12.** stamen (×18), **13.** pistil (× 18), **14.** fruit and calyx (× 7·5), **15.** seed (× 36), **16.** testa (× 180).

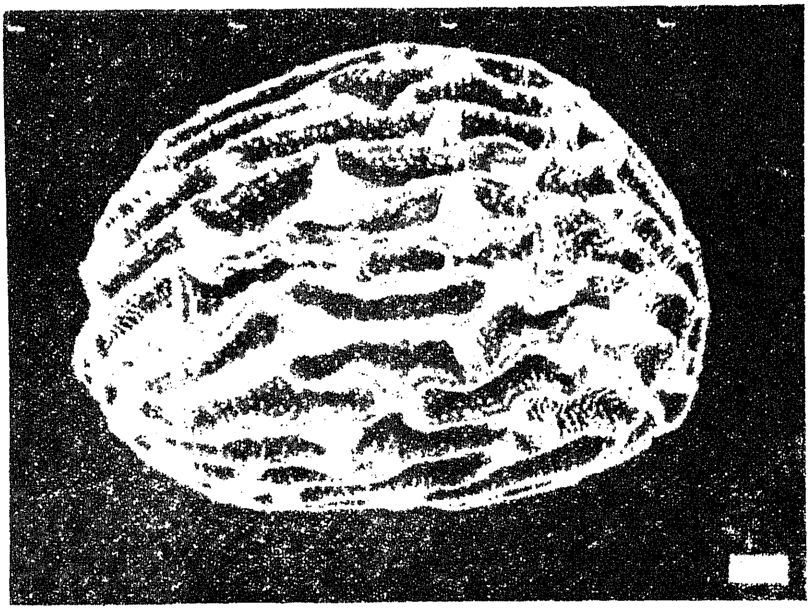

Figure 4. *Utricularia lazulina* P. Taylor. SEM image of seed.

y prominent bigibbous swelling at the base; palate pubescent; spur subulate, rved with apex acute, somewhat longer than and held at an acute angle to the Filaments straight, *ca* 1 mm long; anther thecae distinct. Ovary ovoid, dorsi-ompressed; style distinct and relatively broad; stigma lower lip semicircular, nuch shorter. Capsule ovoid, *ca* 2 mm long, the wall uniformly membranous, by a single longitudinal ventral slit. Seeds obovoid, 0·2–0·3 mm, with a al slightly prominent hilum, distinctly reticulate, the testa thin, microscopi-icose, with cells irregular, slightly elongate (figures 3 and 4).

on. India: Karnataka, South Kanara, in several places about Mangalore! and a ier north on the way to Kollur.

hallow wet soil over laterite and in wet grassland at low altitude. Flowering in August.

ria lazulina P. Taylor, so named for the clear blue colour of the corolla, be nearest to *U. albocaerulea* which also has a blue (rather the usual violet or rolla. However the latter species has larger flowers with a much broader olla lip and the seeds are very different indeed. The seeds of *U. lazulina* are the section, in having the periclinal wall conspicuously verrucose. on under SEM shows that the seeds are in fact covered by a smooth wax layer forms with the contours of the verrucae on the cuticle beneath.

dgements

r thanks Sue Hillier for the line drawings and Martin Cheek for the SEM hs.

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Development of the VAM fungus, *Glomus mosseae* in groundnut in static solution culture

K PARVATHI, K VENKATESWARLU and A S RAO
Botany Department, Nagarjuna University, Nagarjunanagar 522 510, India

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Abstract. The establishment of a mycorrhizal fungus, *Glomus mosseae* in groundnut was studied in a static nutrient solution having 0·25 mM of phosphorus and inoculated with powdered infected groundnut roots or resting spores. Initiation of the mycelial growth in roots was observed after 8 days of contact with the fungal inoculum.

In a second experiment initial concentrations of P in the range of 0·25 to 1 mM resulted in maximum colonization by the fungus and increased production of the plant biomass. Plant growth and VAM development was slightly less in a pot culture without the addition of P to the soil. It is suggested that the static solution culture method can successfully be adopted to determine the requirement of initial levels of essential elements in culture solutions for investigating similar mycorrhizal associations of crop plants.

Keywords. *Glomus mosseae*; groundnut; phosphorus; static solution culture; vesicular arbuscular mycorrhiza.

1. Introduction

The importance of vesicular-arbuscular mycorrhiza (VAM) in the uptake of phosphorus (P) in many plants, especially under low nutrient conditions, has been fully realized in recent years (Gerdemann 1975; Khan 1975; Mosse 1973). Most of the reports of VAM in crop plants are restricted to cool-temperate climates. They seem to indicate a rapid colonization of the plants by the VAM fungi. However, an initial lag in the establishment of mycelium and production of vesicles or spores of *Glomus mosseae* in the roots of groundnut was reported in this laboratory (Rao and Parvathi 1982). In the present investigation, an attempt was made to determine the pattern of colonization of groundnut roots by the fungus and also the influence of phosphorus level in the medium on the establishment of the fungus in a static solution culture.

2. Materials and methods

Clean seeds of groundnut cv TMV-2 were sown in pots containing sterilized sand and watered regularly with sterile water. After 10 days of sowing, the seedlings were carefully removed without damaging the roots and the adhering sand particles washed off with sterile distilled water. A porcelain tray (43·3 × 36·5 × 10 cm) was filled with a mineral salts solution, except for the top 2 cm and covered with a bamboo grid, with gaps of about 1 cm^2 to hold the plants in position. The culture solution employed was essentially the same as that used for rice culture by Ishizuka (1933). Micronutrients (Mn, Zn, Ca, B and Mo) were added according to the formulation of Johnson *et al* (1957). The solution had a P content of 0·25 mM in the form of $NaH_2PO_4 \cdot 2H_2O$ and

the pH was adjusted to 4·8–5. About 10 l of medium was provided for 64 plants in the tray. The inoculum of *Glomus mosseae*, obtained by powdering air-dried, infected groundnut roots and passing the powder through a 2 mm sieve or by collecting the resting spores from field soil infested with the fungus, by a wet-sieving method (Gerdemann and Nicolson 1963), was added to this solution. The inoculum consisted of either 1 g of root powder or 50 spores/1 l medium. The seedlings were placed on the grid so that the roots were completely immersed in the solution. They were firmly supported by placing large pebbles on the grid. The tray was left in open shade in the laboratory (room temperature 27° ± 5°C). The culture solution was aerated 3 to 4 times daily by bubbling air through it.

Plant samples in triplicate were withdrawn at desired intervals. At the end of the 20th day, the entire inoculated medium was drained off from the tray and substituted with an equal quantity of fresh uninoculated medium. A total of 25 one cm segments of root collected from three plants at random were sampled each time and observed for the presence or absence and the nature of mycorrhizal structures, after staining as described earlier (Rao and Parvathi 1982). The average number of vesicles and/or spores per one cm root length and the number of segments showing the fungus were observed to determine the per cent infection.

An experiment was also set up in pots using sterilized lateritic soil (pH 7·8; organic matter, 0·26 %; total nitrogen 0·0616 %; total phosphorus, 0·0045 %) inoculated with powdered roots from infected plants. No external source of P was added to the soil. About 10 seeds were sown in each of the 20 pots prepared this way and the pots were incubated as mentioned above and mycorrhizal development was assessed.

In another experiment, groundnut seedlings raised in sterilized sand were transferred to the bamboo grid placed over a glass tank (16·2 × 6.5 × 18 cm) containing 2 l of the culture solution with different initial concentrations of phosphorus (0, 0·1, 1 and 10 mM). Uninoculated solution with 0·25 mM of P was maintained as the control. About 20 plants were raised in two tanks for each treatment. The sides of the tanks were covered with black paper to avoid algal growth in the medium and incubated in the laboratory.

After 20 days the entire solution was removed and the tanks were refilled with fresh uninoculated nutrient solution with the same concentrations of phosphorus. Subsequently, three plants were taken out at intervals of 5 days and the roots examined for the nature and extent of mycorrhizal formation. At the time of the last sampling, five plants were removed for each treatment, oven-dried to constant weight at 70°C and the weights recorded. The phosphorus content of shoots and roots was determined by the method of Fogg and Wilkinson (1958).

3. Results

The data on colonization of groundnut roots by *Glomus mosseae* under static conditions of nutrient culture are presented in figure 1. The data of the corresponding experiment with sterilized soil are presented in figure 2. In the culture solution, the mycorrhizal fungus appeared in host roots as sparse hyphae only after 8 days of contact with the inoculum. The vegetative growth of the fungus increased progressively up to 18 or 19 days and a sudden development of tiny vesicles was noticed on the 20th day in the medium. In the plants raised in inoculated sterilized soil, vesicles started to develop

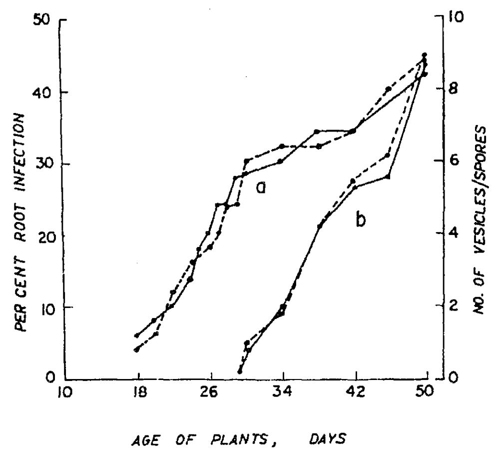

Figure 1. Development of *G. mosseae* in groundnut roots in static solution culture. Ten-day old seedlings raised in sterilized sand transferred to the culture solution. **a.** Per cent mycorrhizal infection and **b.** average number of vesicles and/or spores per cm root. Solid line (——), root powder inoculum and broken line (– – – –), spore inoculum.

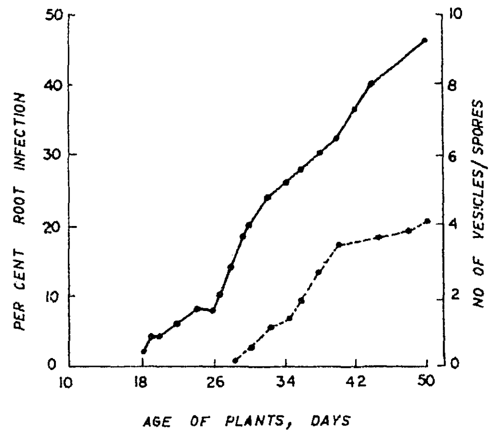

Figure 2. Development of *G. mosseae* in groundnut roots raised in sterilized soil in the presence of root powder inoculum. Solid line (——), per cent mycorrhizal infection and broken line (– – – –), average number of vesicles and/or spores per cm root.

after 28 days. Arbuscules were noticed prior to the appearance of vesicles in the culture solution experiment with spore inoculum and in sterilized soil with powdered root inoculum. With the appearance of vesicles, the arbuscules started to disappear. In all the cases, root infection as well as vesicle formation increased progressively. After 46 days of plant growth, some of the vesicles started developing into thick-walled spores. During the 40-day incubation period in culture solution, about 44 % of the root segments examined were colonized and the vesicle and/or spore formation reached a maximum of 8·9/cm root. In sterilized soil, 46 % of root segments were colonized but

Table 1. Effect of phosphorus level in culture solution on the establishment of *Glomus mosseae* in groundnut roots.

P level in cluture solution (mM)	Incubation (days)[a] 20	25	30	35	Root length (cm)	Total dry wt. (g/plant)	P content (mg/g material) Shoot	Root
Inoculated								
0	12[b]	12	14(0·2)[c]	14(0·4)	10·82	0·93	0·16	0·06
0·1	16(0·2)	20(1·9)	22(2·0)	24(2·6)	13·75	1·35	1·30	0·10
1·0	24(0·6)	28(2·2)	34(3·9)	38(4·5)	17·85	1·76	2·00	1·50
10·0	0	0	0	0	6·25	0·70	10·00	8·00
Uninoculated								
0·25	0	0	0	0	8·34	0·54	1·40	0·30

[a] Ten-day old seedlings grown in sterilized sand were transferred into the culture solution containing inoculum (0·1 % infected root powder, w/v) and incubated further; [b] Per-cent root length infected; [c] Figures given in parantheses represent the average number of vesicles or spores/cm root length.

the maximum number of vesicles and/or spores per cm root length was only 4·1, in the corresponding period.

In the study involving different initial levels of phosphorus in the nutrient solution, root infections were observed even in the plants exposed to inoculum in the medium receiving no phosphorus. However, the development of vesicles was delayed up to 30 days of incubation (table 1). The roots of plants receiving 0·1 and 1 mM of initial P concentrations exhibited comparatively better hyphal growth and the vesicles started developing at the end of 20 days. The plants showed maximum root length and total dry weight at 1 mM of initial P content. There was virtually no mycorrhizal infection at a higher level (10 mM) and this concentration also appeared to be toxic to the plant as seen in reduced dry weight, root length and general appearance. The plants, however, had 1 % P in tops and 0·8 % in roots. The growth of plants raised in uninoculated solution containing 0·25 mM P was less than in the solution without P but inoculated with the fungus.

4. Discussion

In the present investigation, an attempt was made to ascertain the applicability of static conditions of nutrient solution culture for the effective establishment of the VAM fungus, *G. mosseae* in groundnut roots. In the plants raised in nutrient solution as well as in sterilized soil, the fungus appeared only after 8 days of transfer to the solution or after 18 days of sowing, which was confirmed in repeat experiments. The occurrence of only the hyphal phase of the fungus in the roots up to 28 days also clearly shows that the development of the mycorrhizal endophyte in the culture solution with an initial concentration of 0·25 mM of P proceeds in a manner similar to that obtained in soil.

The arbuscules, implicated to be the structures involved in active uptake of P by plants (Mosse 1973) were observed at 28 days of plant growth, prior to the initiation of vesicles. However, the arbuscules were found only when the laboratory temperature

was relatively low (27°C) both in solution culture and in sterilized soil. In a repeat experiment conducted with the solution culture at a higher temperature (32°C), arbuscules did not appear. The development of vesicles after 28 days, however, was uniform in all cases. This finding extends our earlier report that, in the semi-arid soil conditions as exist in this locality, a lag of 3 to 4 weeks occurs before the vegetative mycelium of *G. mosseae* in groundnut roots is transformed into vesicles (Rao and Parvathi 1982). On the other hand, in some Australian soils, maximum number of entry points on the roots of *Medicago truncatula* and the development of arbuscules occurred on 8th day at 12–16°C (Smith and Bowen 1979). However, they reported that, for *Trifolium subterraneum* there was a delay of 8–10 days for the entry of the fungus even at these temperatures. In another study, Schenck and Schroder (1974) found that development of arbuscules and per cent infection of soybean roots declined above 30°C with no infection at 41°C. The total absence of arbuscules at 32°C and a relatively long lag phase of 28 days in the development of vesicles in the present study are perhaps due to the higher temperatures prevailing under our experimental conditions.

In soil-grown plants, P uptake is commonly limited to a narrow zone of soil adjacent to the root and to eliminate this limitation a flowing culture technique with very low P concentration was developed by Howeler *et al* (1982) to establish an effective endomycorrhizal association on cassava. The present investigation indicates that a much simpler method of static solution culture providing higher level of P can be successfully used for mycorrhizal development. The limitation of available phosphorus that occurs in soil would not be a limiting factor in a liquid medium as the concentration would adjust itself through diffusion.

To ascertain the influence of initial P concentration on the growth of the plants and for the development of the fungus in the solution culture, three different levels (0·1, 1 and 10 mM) of P were employed. The ten-day old seedlings transferred to the culture solution containing the inoculum continued to grow, although there was no added P in the medium. They had 1·1 mg P/g plant material at the time of transfer which got reduced to 0·16 mg P/g shoot material by 35 days. Apparently, the P already present in the seedlings was sufficient for further growth. Under these conditions the mycorrhizal fungus showed poor vegetative growth and a delay in the development of vesicles. But the degree of infection and the development of vesicles and/or spores was more or less the same in 1 mM level of P in culture medium as at 0·25 mM of P in the previous experiments. Hence, a range of 0·25 mM to 1 mM of initial P in the culture medium may be considered adequate for the growth of the plant and for mycorrhizal development.

The flowing culture techniques developed by Howeler *et al* (1981) and Macdonald (1981) are more elaborate and require large quantities of nutrient solution. The present technique, which is simpler than the flowing culture technique, needs only daily aeration and a change in the nutrient solution after 20 days, while indicating the response of the plant and the fungus clearly.

Acknowledgement

The authors are grateful to Dr J W Gerdemann and Dr J M Trappe for confirming the identity of the mycorrhizal fungus.

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Carbohydrate changes induced by temperature and vitamins in green gram (*Vigna radiata* L. Wilczek) seedlings

P GOPALA RAO and G SUDARSANAM
Department of Botany, Sri Venkateswara University, Tirupati 517 502, India

MS received 23 June 1983

Abstract. Temperature and vitamin-induced changes in root elongation are closely associated with changes in non-reducing sugar content in particular. Thiamine enhanced the reducing sugar content of the shoot and reduced that of the root at normal temperature. In contrast, elevated temperature caused a significant reduction in the reducing sugar content of the shoot and increased that of the root. Thiamine at elevated temperature showed a synergistic effect in decreasing the sugar content of the shoot and increasing it in the root. Although riboflavin could not enhance the reducing sugar content of the shoot at normal temperature, at elevated temperature its response was quite similar to that of thiamine. These differences were partly associated with amylase activity of the root and the shoot. Vitamin treatment preceded by elevated temperature showed synergistic effect with respect to non-reducing sugar content by increasing it in the shoot and the root. The role of vitamins is discussed.

Keywords. Thiamine; riboflavin; elevated temperature; reducing and non-reducing sugar content; root and shoot elongation.

1. Introduction

The role of vitamins in higher plants is far from clear, although their role as coenzymes is evident. Some vitamins do have other roles in the biochemical processes of the organisms. Evidence has accumulated that vitamins of the B group exert significant effect on growth, development and yield of certain crops (Filimovo 1967; Bogdonova 1965; Polimbetova *et al* 1969). The mechanism by which temperature controls growth is not known. The present study has been designed to understand the role of vitamins with an alteration in the temperature regime in relation to changes in carbohydrates and associated enzymes. Carbohydrates form the principal energy sources for plant growth. Temperature interactions with growth regulators and endogenous gibberellin-like activity during seed stalk elongation in carrot roots were studied by Hiller *et al* (1979).

2. Materials and methods

Seeds of green gram (*Vigna radiata* L. Wilczek) were surface-sterilized with 0.1 % formic acid for 30 min and washed thoroughly in tap water and soaked in distilled water for 24 hr at room temperature (28°C ± 2) in 6 inch dia petridishes. The germinated seeds were then kept in a BOD incubator for 18 hr at 40°C to maintain an elevated temperature regime. The seedlings were then taken out and treated with riboflavin (10 mg l^{-1}) and thiamine hydrochloride (20 mg l^{-1}) after a preliminary screening for optimal growth promoting concentrations. The vitamin treatment was given only for

24 hr after heat treatment and thereafter the seedlings were allowed to grow in distilled water at 28°C during the experimental period. Desiccation was prevented by supplying water at 6 hr intervals to the seeds during incubation at elevated temperature. Distilled water controls were maintained separately at normal temperature. The seedlings were allowed to grow in petridishes for 8 days under continuous illumination of 150 $\mu E\,m^{-2}\,sec^{-1}$ and sugars and enzyme activity were determined at 2-day intervals in the root and the shoot separately. Reducing sugars were estimated according to Snell and Snell (1957). The non-reducing sugar content was estimated using the method of Scott (1960). The α-amylase was assayed according to Bernfeld (1955). The length of the shoot and the root was separately measured. The pH of the vitamin solutions was maintained at 6.5 with a mild alkali.

3. Results and discussion

There was a significant increase in reducing sugar content of the root at elevated temperature (figure 1). The decrease in the content of reducing sugars in the shoot was quite significant. These results showed some association with amylase activity (figure 2) *i.e.* increased activity in the root and decreased activity in the shoot at 40°C. An increase in reducing sugar content of the root was associated with an increase in root elongation (table 1). The increase in root elongation at 40°C was 92.3, 144.5, 87.5 and 93.5 % on the second, fourth, sixth and eigth days respectively. An increase in root elongation was associated with a decrease in shoot elongation at 40°C. The non-reducing sugar content of the root (figure 3) showed a marked increase at elevated temperature. The role of carbohydrates in root elongation and root formation in cuttings has been discussed for many years (Kraus and Kraybill 1918; Moore *et al* 1972). Nanda and Jain (1971) and Nanda and Dhaliwal (1973) showed that root elongation requires a balanced ratio of sucrose and auxin. They also stated that auxin either promoted or inhibited root growth and initiation depending on the glucose concentration. In the present study increased root elongation at elevated temperature appears to be a consequence of an increased level of reducing as well as non-reducing sugar content. This might be either due to increased translocation from the shoot to the root or an increased activity of amylase in the root at 40°C. There is evidence of the translocation of sucrose from the mature tissues to the apex and its release from the phloem strands and diffusion *via* intercellular spaces and walls to the meristem (Street 1966).

Thiamine at higher concentration (20 $mg\,l^{-1}$) promoted root elongation only up to the fourth day followed by a decline when compared to the control seedlings (28°C). At lower concentration (10 $mg\,l^{-1}$) it was not promotive either to the root or to the shoot (table 1). Thiamine promoted the shoot elongation also at higher concentration with a concomitant increase in reducing sugar content at 28°C. However, at 40°C the reducing sugar content of the shoot was reduced and that of the root was enhanced significantly (figure 1) showing association with shoot and root elongation respectively. The response of elevated temperature followed by thiamine treatment appears to be synergistic in this connection *i.e.* in increasing the reducing sugar content of the root.

Riboflavin at lower concentration (10 $mg\,l^{-1}$) promoted root elongation only up to the fourth day followed by a decline when compared to the control seedlings (table 1). At higher concentration (20 $mg\,l^{-1}$) it was even inhibitory to root elongation. Even the promotion at lower concentration was quite marginal. The response of elevated

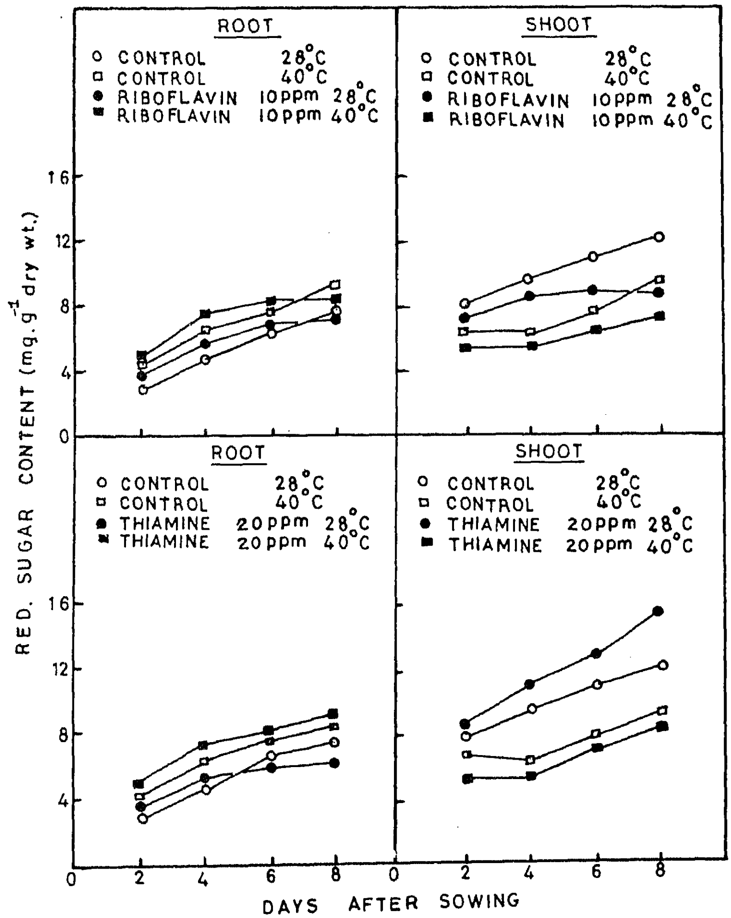

Figure 1. Temperature and vitamin effect on reducing sugars.

temperature followed by riboflavin ($10\,mg\,l^{-1}$) was better than that at normal temperature up to the fourth day, but it was relatively less with thiamine. However, the reducing sugar content was markedly high (figure 1). Elevated temperature (40°C) caused a significant increase in amylase activity of the root as well as that of the shoot (figure 2). At 28°C, riboflavin and thiamine enhanced the amylase activity of the shoot in particular. The raise in enzyme activity of the shoot at 40°C was more prominent on the second day while it continued up to the sixth day in the root. Elevated temperature followed by vitamin treatment resulted in a significant increase in enzyme activity of the shoot on the second day followed by a decrease even below the level of seedlings grown at 28°C. This initial increase was much less in the root. Thus, these changes in α-amylase activity were not commensurate with the changes in reducing sugar content since this is not the only enzyme that is responsible for the formation of reducing sugars.

The influence of elevated temperature and vitamins was more striking on the non-

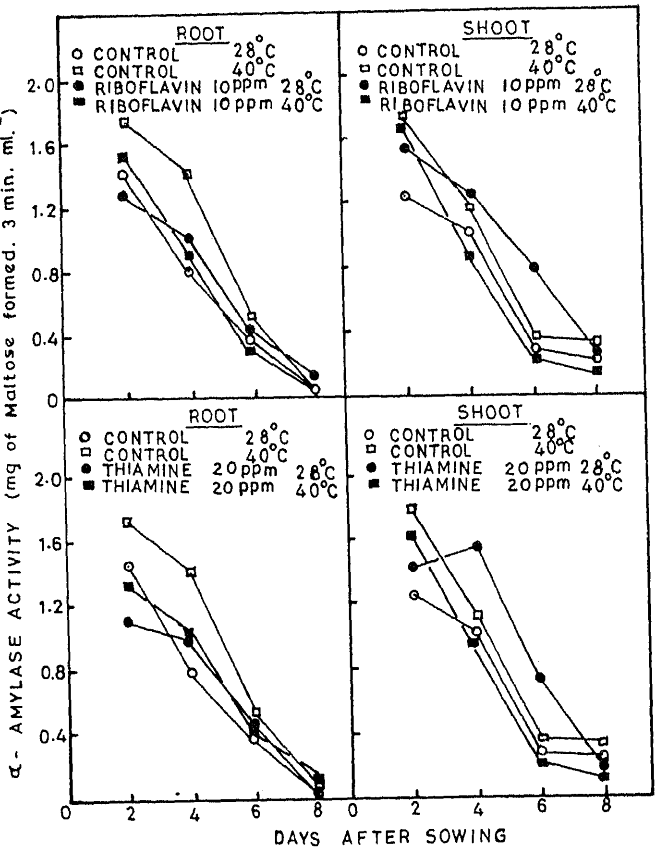

Figure 2. Temperature and vitamin effect on α-amylase.

reducing sugar content (figure 3). At 28°C, riboflavin was more effective in increasing the non-reducing sugar content of the root as well as that of the shoot when compared to thiamine. At 40°C, the effect of these vitamins was still marked. In the shoot, however, elevated temperature caused a marked decrease in the sugar content up to the sixth day followed by an increase over that of the normal temperature. This is because of a steep fall on the eighth day at 28°C.

Thus, it is inferred that elevated temperature succeeded by vitamin treatment caused changes in elongation of the root as a sequel to changes in non-reducing sugar (may be sucrose) content in particular. The response exhibited by reducing sugar content was relatively less. At 40°C, the decrease in shoot elongation was associated with a decrease in non-reducing sugar content at least up to the sixth day, when compared to that at 28°C. Further, it is to be noted that at 40°C, the decrease in shoot elongation and an increase in non-reducing sugar content with vitamin treatment were not associated. Thus the results indicate that vitamins increase the non-reducing sugar content irrespective of elongation growth, when temperature was raised prior to their

Table 1. Effect of elevated temperature and vitamins on the seedling growth of green gram.

Days after sowing		Control		Thiamine (28°C)		Riboflavin (28°C)		Thiamine (40°C)		Riboflavin (40°C)	
		(28°C)	(40°C)	(10 ppm)	(20 ppm)	(10 ppm)	(20 ppm)	(10 ppm)	(20 ppm)	(10 ppm)	(20 ppm)
2	Root	3·38 ±0·49	6·50 ±0·58	3·15 ±0·43	3·90 ±0·26	3·60 ±0·24	3·00 ±0·62	4·10 ±0·75	4·60 ±0·56	3·80 ±0·60	3·20 ±0·39
	Shoot	10·40 ±0·63	8·00 ±0·54	10·90 ±0·28	12·10 ±0·39	11·50 ±0·58	11·48 ±0·74	7·38 ±0·37	8·90 ±0·53	7·76 ±0·21	6·80 ±1·06
4	Root	3·84 ±0·14	9·40 ±0·96	3·16 ±0·10	4·10 ±0·18	3·92 ±0·32	3·20 ±0·18	4·28 ±0·12	6·20 ±0·67	5·20 ±0·60	4·24 ±0·77
	Shoot	20·10 ±0·98	17·40 ±0·60	20·14 ±0·39	21·80 ±0·77	20·40 ±0·43	19·98 ±0·88	19·70 ±0·58	18·60 ±0·93	19·12 ±0·87	18·52 ±0·46
6	Root	5·65 ±0·76	10·60 ±0·57	4·20 ±0·20	4·98 ±0·29	4·62 ±0·36	4·10 ±0·25	5·85 ±0·34	6·30 ±0·34	5·60 ±0·24	5·20 ±0·47
	Shoot	22·50 ±0·49	19·40 ±0·61	22·00 ±0·49	24·40 ±0·82	23·50 ±0·58	20·10 ±0·52	21·30 ±0·55	20·10 ±0·88	21·50 ±0·67	19·86 ±0·53
8	Root	6·20 ±0·81	12·00 ±0·63	4·60 ±0·43	5·90 ±0·24	5·10 ±0·58	4·40 ±0·74	6·10 ±0·32	7·20 ±0·39	6·10 ±0·46	5·20 ±0·06
	Shoot	24·20 ±0·57	20·20 ±0·81	23·80 ±0·51	26·10 ±0·28	25·20 ±0·39	24·10 ±0·53	22·20 ±0·77	22·40 ±0·91	23·20 ±0·75	21·60 ±0·91

Note: Extension growth is expressed as length in cm. Each value is a mean of 12 replications (±SE).

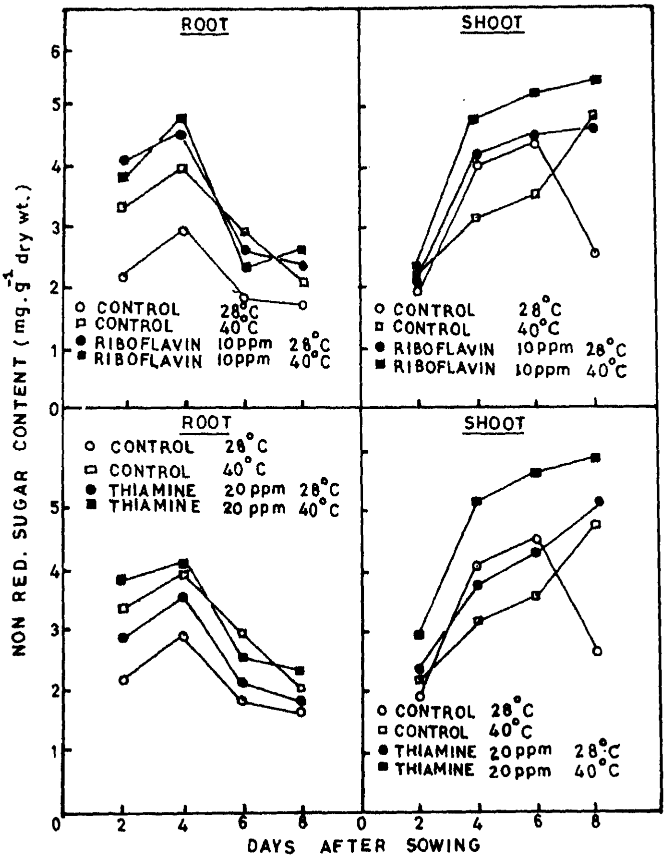

Figure 3. Temperature and vitamin effect on non-reducing sugars.

treatment. Apparently this may involve an enzyme activation associated with non-reducing sugar formation. Vitamins appear to mimick gibberellic acid in this regard *i.e.*, in increasing reducing and non-reducing sugar content. Gibberellic acid activates α-amylase as well as invertase activity (Paleg 1965). At normal temperature, the shoot elongation was better and uniform with vitamin treatments *i.e.*, at lower concentration of riboflavin and higher concentration of thiamine, when compared to root elongation. At 40°C, the shoot elongation was reduced and that of the root enhanced with vitamin treatment which might be due to an enhanced translocation of sugars from the shoot to the root.

Acknowledgement

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Systematics of genus *Lygodium* Sw. (Lygodiaceae) in India

S SINGH and G PANIGRAHI

Botanical Survey of India, Howrah 711 103, India

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Abstract. *Lygodium* Sw. belongs to the monogeneric family Lygodiaceae Presl. *s.s.* and is comprised of about 40 species in the world flora. Out of these 10 species are from India. *L. giganteum* Tagawa and Iwat. and *L. mearnsii* Copel. are new records while *L. longifolium* (Willd.) Sw., *L. circinnatum* (Burm. f.) Sw., *L. polystachyum* Wall. ex Moore and *L. altum* (Clarke) v.A.v.R. are poorly represented amongst the Indian collections in herbaria. A key is provided for diagnosing the species from India along with the correct nomenclature of each taxon.

Keywords. Lygodiaceae; *Lygodium*.

1. Introduction

Beddome (1883, 1892) recorded five species/varieties of *Lygodium* L. from India: *L. circinnatum* (Burm. f.) Sw., *L. microphyllum* (Cav.) R. Br., *L. flexuosum* (L.) Sw., *L. japonicum* (Thunb.) Sw. and *L. flexuosum* (L.) Sw. var. *alta* C. B. Clarke. Mehra and Bir (1964) listed *L. flexuosum* and *L. japonicum* from Darjeeling-Sikkim Himalayas. Panigrahi and Dixit (1967), described and illustrated the four species *sensu* Beddome and listed the chromosome numbers of 10 species investigated up to 1966. Although *L. longifolium* (Willd.) Sw. (1803) was originally described from South India, neither Beddome nor Panigrahi and Dixit (1967) included the taxon in their treatment; Holttum (1959) treated *L. mearnsii* Copel. from the Philippines as conspecific with *L. japonicum* Sw. and reported one more species: *L. polystachyum* Wall. ex Moore as extending its range to Assam. *L. salicifolium* Presl was originally based on plants from India and Bangladesh. However this taxon was not recognised in the Indian herbaria correctly until Holttum (1959) reported the extension of its range to Assam. Thus the taxonomy of *Lygodium* is in a state of flux. The present studies based on field observations and on the materials deposited in CAL and ASSAM clearly show that the genus is represented in India by 10 species.

2. Materials and methods

A large number of specimens from all over India and abroad (in CAL and ASSAM) have been examined. For correct identification, materials, protologues, photographs, illustrations and microfiche-photographs of 'Types' and/or of authentic specimens as available at CAL were consulted.

3. Systematic account

Lygodiaceae Presl, Suppl. Tent. Pterid. 98. 1845; Pichi Sermolli in Webbia 31(1): 327. 1977, et Taxon 30(1): 166. 1981.

Type genus: Lygodium Sw. in *J. Bot.* (Schrader) 1800(2): 7, 106. 1801 *nom. cons.*

Terrestrial, with rhizome short-to long-creeping, on or under the surface, apex covered with rigid multiseptate hairs. *Fronds* in juvenile plants erect, once or twice forked or dichotomously branched with lamina palmatilobed; the fronds of the older plants grow further by the elongation of rachis to an unlimited growth; stipe with one vascular bundle; *rachis* (or main rachis) branch into unequal dichotomies, the dominant branch elongates, twists and climbs up by branching similarly repeatedly upwards at regular intervals; recessive branch develops into palmate leaflets or pinnae; flexible, stout or plastic, glabrous or hairy, dorsal surface flat; with very narrow wing of raised edges (except *L. polystachyum*), ventral and lateral surfaces terete, wings interrupted, all sub-branch-rachises similarly winged; *Pinnae* forked, palmati-lobed; *Primary rachis-branch* short, hardly developed or to 10 mm long, forked, glabrous or pubescent, dorsal surface flat (except in *L. polystachyum*), ending into dormant apex bearing brown to black-brown septate, pointed hairs; sometimes the dormancy is overcome if the apex is injured, each branch of the fork of the primary rachis-branch develops into long secondary rachis-branches, these may either directly bear stalked or subsessile pinnules or may further give rise to tertiary and quaternary rachis-branches, morphology similar to primary or secondary rachis-branches; junction beyond may be jointed or continuous; *pinnules* simple to lobed to bi-tripinnate, sessile to stalked, stalks articulated (or jointed) or nonarticulated to secondary rachis-branches or to the pinnule-lamina; margin entire to crenate; veins 1–3 times forked, very oblique, free, if reticulate then without free included veinlets (not in Indian taxa); surfaces may be glabrous or pubescent. *Sorophores* at the margin, usually with 2–7 sporangia or more, acropetal, alternate, in two rows, one on each side of the vein; indusium individual, attached along the vein and opening forwards; edges entire or fringed or hairy; *sporangia* bullet-shaped or pear-shaped, annulus at one end in a single row of elongated thickened cells, bursting longitudinally or irregularly, arising at the margin but becoming superficial due to subsequent extra-marginal growth; *spores* pale green or looking brown in dried specimens, trilete, variously sculptured but perispore absent.

Monogeneric (sensu Pichi Sermolli 1981)

Lygodium Sw. in *J. Bot.* (Schrad.) 1800(2): 7, 106. Oct.-Dec. 1801; *nom. cons.*

Lectotype: *Lygodium scandens* (L.) Sw. l.c. *non sensu* Sw. (≡ *Ophioglossom scandens* L. Sp. Pl. 2: 1063. 1753 *p.p.* ≡ *O. flexuosum* L. = *Lygodium flexuosum* (L.) Sw. *p.p.* excl. syn. *O. circinnatum* Burm. f. (lectotype selected by Underwood in Mem. Torrey Bot. Cl. 6(4): 280. 1899. (see Panigrahi and Singh 1983).

4. Key to the species

1a. Primary rachis-branches hardly distinct or up to 3 mm long; rhizome short-creeping or suberect.

2a. Secondary rachis-branches pinnate with 10–15 pairs of pinnules; axes terete and wingless throughout; sterile pinnules pinnatifid throughout . . . . 9. *L. polystachyum*

2b. Secondary rachis-branches pinnate with 2–6 pairs of pinnules or branches dichotomously; axes flat on dorsal side and winged; sterile pinnules simple pinnate at base or palmate

3a. Secondary rachis-branches regularly pinnate, with 3–6 pinnules on either side

4a. Basal pinnules the largest, the succeeding ones gradually reduced; basal pinnules stalked becoming sessile and subsessile and thereafter adnate upwards . . . . 3. *L. flexuosum*

4b. Pinnules ± all equal and stalked

5a. Stalks articulated at their junction with lamina which falls off leaving the naked stalks; occasionally the basal pinnules of secondary pinnae lobed to pinnate; dormant apex hairs without swollen bases; costules ± raised only to the mid lamina . . . . 10. *L. salicifolium*

5b. Stalks not articulated with the lamina; basal ultimate pinnules at most with one lobe; dormant apex hairs with swollen bases; costules prominently raised below and conspicuous to the tip of the lamina . . . . 1. *L. altum*

3b. Secondary rachis-branches once or twice-forked or dichotomously branched, sometimes sub-pinnate with 1–2 tertiary pinnules.

6a. Sterile pinnules finely serrate or crenate, margin not much thickened, veins ending into teeth; dormant apices of primary branch not sunk; hairs of dormant apex with swollen bases; spores coarsely or irregularly warty verrucose . . . . 6. *L. longifolium*

6b. Sterile pinnules entire, margin thickened by the joining of the veins; dormant apices sunk; bases of hairs of dormant apices not swollen; spores finely or evenly verrucose . . . . 2. *L. circinnatum*

b. Primary rachis-branches 3–10 mm long; rhizome generally long-creeping, or short-creeping.

7a. Fertile secondary branches either simple pinnate, or if more, then finely dissected; rhizome long-creeping and fronds distantly placed.

8a. Secondary branches simple pinnate, pinnules deciduous, basal pinnules occasionally lobed; pinnule stalks thickened at the apex; spores with raised reticulum on the upper side . . . . 8. *L. microphyllum*

8b. Secondary rachis-branches bipinnate or more pinnate in fertile pinnae; pinnules palmately-lobed and persistent; pinnule stalks not thickened; spores finely verrucose . . . . 5. *L. japonicum*

7b. Fertile secondary rachis-branches pinnate with pinnules palmately lobed at base or sometimes these lobes are free; rhizome short-creeping and fronds close together

9a. All junctions beyond the secondary rachis-branch jointed or articulated; spores smooth . . . . 4. *L. giganteum*

9b. None of the junctions is joined or articulated, (axes continuous in growth at every junction); spores finely verrucose . . . . 7. *L. mearnsii*

5. Description of the species

1. ***Lygodium altum*** (C. B. Clarke) v.A.v.R., Malayan Ferns 114. 1908.

Lygodium flexuosum var. *alta* C. B. Clarke in *J. Linn. Soc. Bot.* **25** 101. t. 44. 1890; Beddome, Suppl. Handb. Ferns Br. India 108 1892.

Type: India, West Manipur (Muneypore) *Clarke* 42331.
Lygodium flexuosum sensu Panigrahi and Dixit in *Proc.* Aut. Sch. Bot. Mahabaleshwar 217 1967 *p.p. includo L. flexuosum* var. *alta* C. B. Clarke *pro syn. tantum.*

Rhizome short-creeping. *Fronds* very long, 70–85 cm wide, *rachis c.* 2 mm across, *pinnae* each half 35–42 cm long, 30–40 cm broad, deltoid to oblique deltoid, tripinnate; *primary rachis-branches* indistinct, dormant apex hairs up to 2 mm long, blackish-pale, septate with swollen base intermixed with/without swollen base; *secondary rachis-branches* 6–20 cm long, wing distinct, dorsal flattened surface with simple or septate, hyaline to brown hairs; pinnate; *pinnules* largest lateral 20–25 cm long, 2·3–3·5 cm wide in middle, 2–4 pairs, basal ones with or without lobes, terminal pinnae forked or deeply lobed, 23–28 cm long, 2–3 cm broad, all stalked, stalk 1–1·5 cm long in basal, gradually reducing upwards, winged, glabrous on both surfaces or a little bit pubescent on dorsal side as on sec. rach.-br., pinnule lamina base cordate, sterile margin subcrenate, mid vein pubescent (or with scattered hairs) on upper surface; lower surface glabrous; pinnule-stalk nonarticulate, veins free, 3–4 times forked, prominent; glabrous, shining, texture subcoriaceous. *Sorophores* 2–6 mm long, 1·5 mm broad, indusium not hairy; spores 75-75-75 × 82-82-82 μm, pale brown, tuberculate or verrucose (figures 1–4).

Specimens examined: India: Meghalaya, K. & J. Hills. Thleu Syrryngam, 100 m, 6 Dec. 1915, *Kanjilal* 6320 (ASSAM); Arunachal Pradesh, Subansiri District, Itanagar, *ca.* 450 m, 21 Dec. 1970, *G. D. Pal* 70325 (BSI Herb. Itanagar). Burma: Salween District. Papun, Jan. 1912, *Meebold* 16969 (CAL). This specimen has got brown, long, septate, pointed hairs on the upper surface of pinnules and more so at the base of the sorophores on both surfaces.

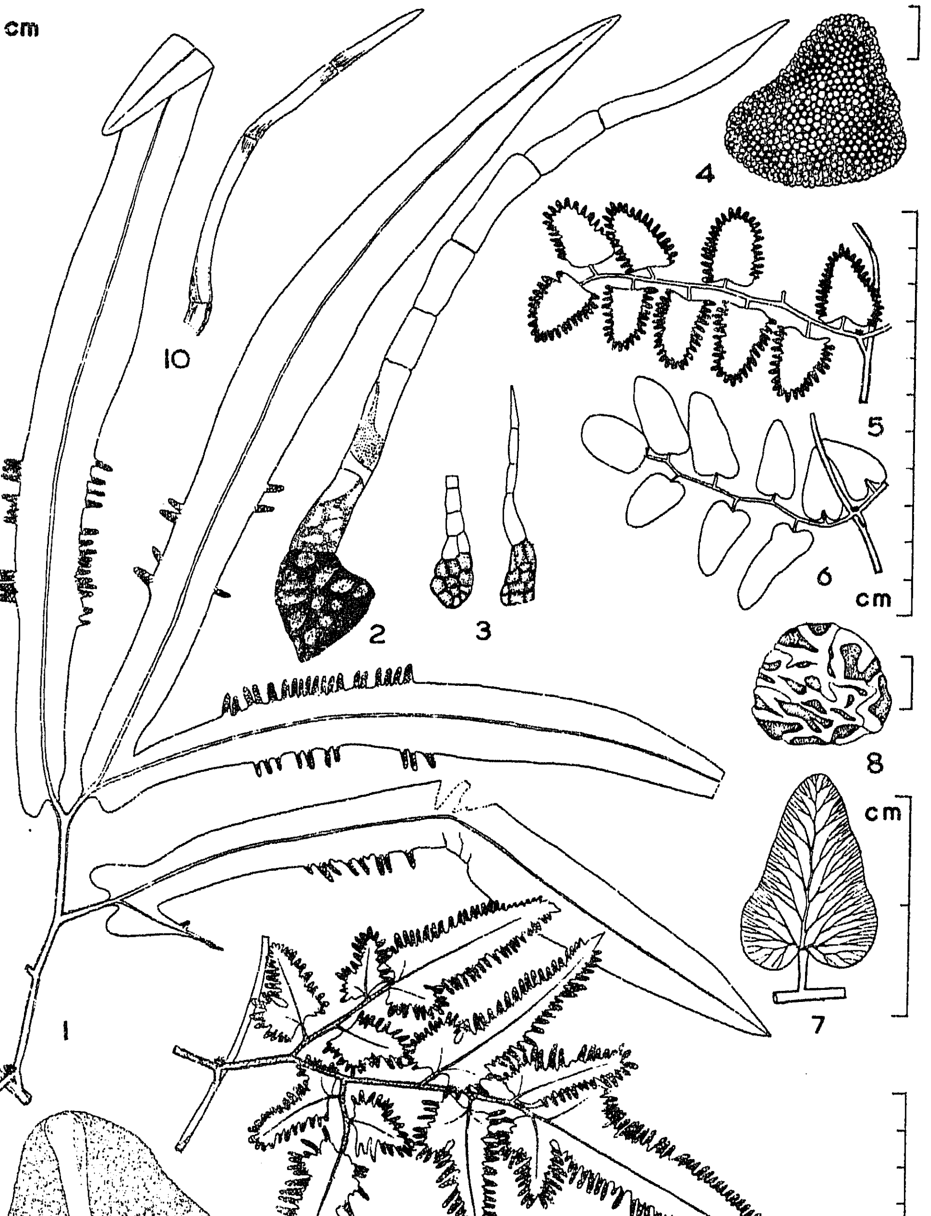
Distribution: India (Meghalaya, Manipur, Arunachal Pradesh) and Burma.

2. ***Lygodium circinnatum*** (Burm. f.) Sw. Syn. Fil. 153. 1806; Beddome, Handb. Ferns Br. India 455. 1883 *p.p. excl.* t. 281 et *L. dichotomum* Bedd. Ferns S. India t. 62. 1863 *pro syn*; Alston and Holttum in Reinwardtia 5: 20. 1959; Holttum in Fl. Malesiana Ser. II. Vol. 1(1): 59. f. 5d. 14. 1959; Panigrahi and Dixit *op. cit.* 216. f. 1. *p.p. excl. spec.* Khasia Hills.

Ophioglossum circinnatum Burm. f. Fl. Ind. 228. 1768.

Type locality: Habitat in Amboina and Java (*Burman s.n.* – G, Vide Holttum l.c. – not seen)
Lygodium dichotomum (Cav.) Sw. Syn. Fil. 154. 1806 *non* Beddome (1863).
Ugena dichotoma Cav. Ic. Descr. Pl. 6: 74. t. 594. f. 2. 1801.

Type: "Marianis et Philippines . . . . " (*Nee s.n.*)
Lygodium flexuosum (L.) Sw. J. Bot. in (Schrad.) 1800 (2): 106. 1801, *p.p. incl. syn., excludo typo.*



Figures 1–11. **1–4.** *L. altum* (1,3,4-*Kanjilal* 6320, 2-*Meebold* 16969); **5–8.** *L. microphyllum* (*R S Rao* 20036); **9–11.** *L. flexuosum* (*Nair* 40657); **1, 5, 6, 9.** parts of pinnae; **2, 3, 10.** hair of dormant apices; **7.** pinnule; **4, 8, 11.** spores (scale = 25 μm).

Rhizome short-creeping, bearing stipes very close. *Fronds* very long, 35–50 cm broad, linear, juvenile fronds once dichotomous, each branch bearing a pedato-palmatisect leaflet, lobes usually 6; *rachis ca* 3 mm across, stout, glabrous; *pinnae* each half 18–25 cm long, 15–25 cm broad, once forked or sometimes dichotomously branched, palmatisect; *secondary rachis-branches* 2–5 cm long, glabrous, once forked, each forked branch-bearing trilobed pinnules, all lobes adnate at base, innermost not forked subequal; *ultimate lobes* (sterile) 1·5–2·3 cm broad, elliptic, entire, and thickened; midvein with a few long, brown, septate, pointed hairs on the lower surface; veins once or twice forked, jointed with each other forming thick margin, veins and margin paler than lamina, the lobes broadest in the middle and narrowing towards both ends; (fertile) twice or thrice dichotomously branched, each branch with 3–4 deeply lobed pinnules, 7–10 mm broad; veins once forked, texture subcoriaceous. *Sorophores* 2–5 mm long, *spores* 72-72-72 × 82-82-82 μm, pale, tuberculate or evenly verrucose (figures 35–39).

Specimens examined: *India: S. Andaman*, Dhani Khari-Hilly Jungle, 30 Sept. 1892 *King's collectors s.n.* Acc. No. 26507; Dundas Point-Hill Jungle, 18 April 1895, *King's collector s.n.; N. Nicobars*, Katchal Island, Jhoola, 21 Feb. 1977, *Chakraborty* 5276; Andamans, Betpur, 23 July 1974, *Bhargava* 1852 (CAL).

Distribution: India (North India, Beddome 1883); N.E. India (Holttum 1959), Andaman and Nicobar Islands, Sri Lanka, Bangladesh, Burma, Southern China, Thailand, the New Hebrides and Solomons; throughout Malaysia.

3. ***Lygodium flexuosum*** (L.) Sw. in J. Bot. (Schrader) 1800(2): 106. 1801 *p.p. excludo syn.*; Beddome, *op. cit.* t. 63; Alston and Holttum, *op. cit.*: 15; Holttum *op. cit.*: 53. f. 9e-f.; Panigrahi and Dixit, *op. cit.*: 217 *p.p. excl.* var. *alta* C. B. Clarke.

Ophioglossum flexuosum L. Sp. Pl. 2: 1063. 1753. LT.: Ceylon, Herb. *Harmann* 375 (BM) (selected by Alston and Holttum *l.c.*)
Ophioglossum scandens L. Sp. Pl. 2: 1063. 1753. *p.p.* Type. LT.: Ceylon, Herb. *Hermann* 374 (BM).
Lygodium scandens (L.) Sw. in Journ. Bot. (Schrader) 1800(2): 106. 1801. *p.p. incl. lecto typo tantum.*

Rhizome short-creeping. *Fronds* very long, 25–40 cm broad, juvenile fronds once or twice dichotomous, each branch deeply palmately lobed, almost equal at the base, the whole leaflet cordate, serrate, and sometimes crenately lobed, acute; *rachis ca* 2 mm across or more, dorsal surface puberlous; *pinnae* each half 10–30 cm long, 10–30 cm broad, ovate to deltoid, tripinnate to quadripinnatifid; *primary rachis-branches* indistinct or rarely to 2 mm long, dormant apex bearing hairs 1–1·5 mm long, brown, septate, pointed, dorsal surface puberulous; *secondary rachis-branches* 5–22 cm long, narrowly winged, hairy or puberulous; *pinnules* 3–18 cm long, 1–6 cm broad, ovate-oblong to deltoid, or oblique deltoid, base cordate or auricled and lobed, basal ones largest and stalked becoming sessile upwards, stalks 5–10 mm long, apical smaller and sessile, terminal sessile or stalked simple to lobed to pinnate, in the forms more than 10 cm long then with 2–3 pairs of free pinnule lobes, ultimate segments 1–4 cm long, 1–3 cm broad; these basal pairs stalked; midveins hairy; hairs simple to septate, veins hairy. *Sorophores* 2–10 mm long, *ca* 1 mm broad, *spores* 80-80-80 × 100-100-100 μm, pale, coliculIate or finely verrucose (figures 9–11).

cimens examined: in CAL. *India: Uttar Pradesh* Bank of Sarju River, Kumaon, *ca* m. 10 Aug. 1808, *Strachey and Winterbottom s.n.*; Upper gangetic plains, Kheri ıdh) 1 May 1898, *Inayat* 22931; *Madhya Pradesh*, Bilaspur, Lamni, Feb. 1972, *ıigrahi* 15400; *Goa*, Santa Cruez, 8 Nov. 1962, *Ahuja* 84439, *Karnataka* South ıara, Sitanandi, 6 Nov. 1960, *Arora* 3348; *Kerala*, Travancore State, Aug–Sept. 1913, *der and Ramaswami* 380; Tamil Nadu, Rampu Hills, 25 Sept. 1920, *Narayanswami* *Andhra Pradesh*, Srikakulam District, Salur-Jeypore Road, 700 m, 10 Sept. 1962, *akrishnan* 1065; *Orissa*, Bhitarakanika, 6 Feb. 1961, *Panigrahi* 23729; Koraput, 00 m, 18 Sept. 1970. *Nair* 40657 and Kasipore, Sipijeda, 925 m, 11 Nov. 1973, *Nair* 65; *West Bengal*, Murshidabad, Kalyanpur, Kandi, 14 Oct. 1965, *Guha Bakshi* 177; *ar*, Champaran District, Gobardhana, c. 187 m, 15 Nov. 1963, *Shetty* 321; *Sikkim*, *ızumdar and Dutta* 176; *Assam*, Kamrup District, Rajapara, 16 June 1964, *A. S. Rao* 74; Nowgong District, Sonuikushi R. F. 18 Aug. 1964, *Balakrishnan* 39207; *Tripura*, ıghmun, *ca* 750 m, 23 Jan. 1962, *D. B. Deb* 27090; *Andamans Islands*, 21 Jan. 1904, *in's collector* 98 (CAL).

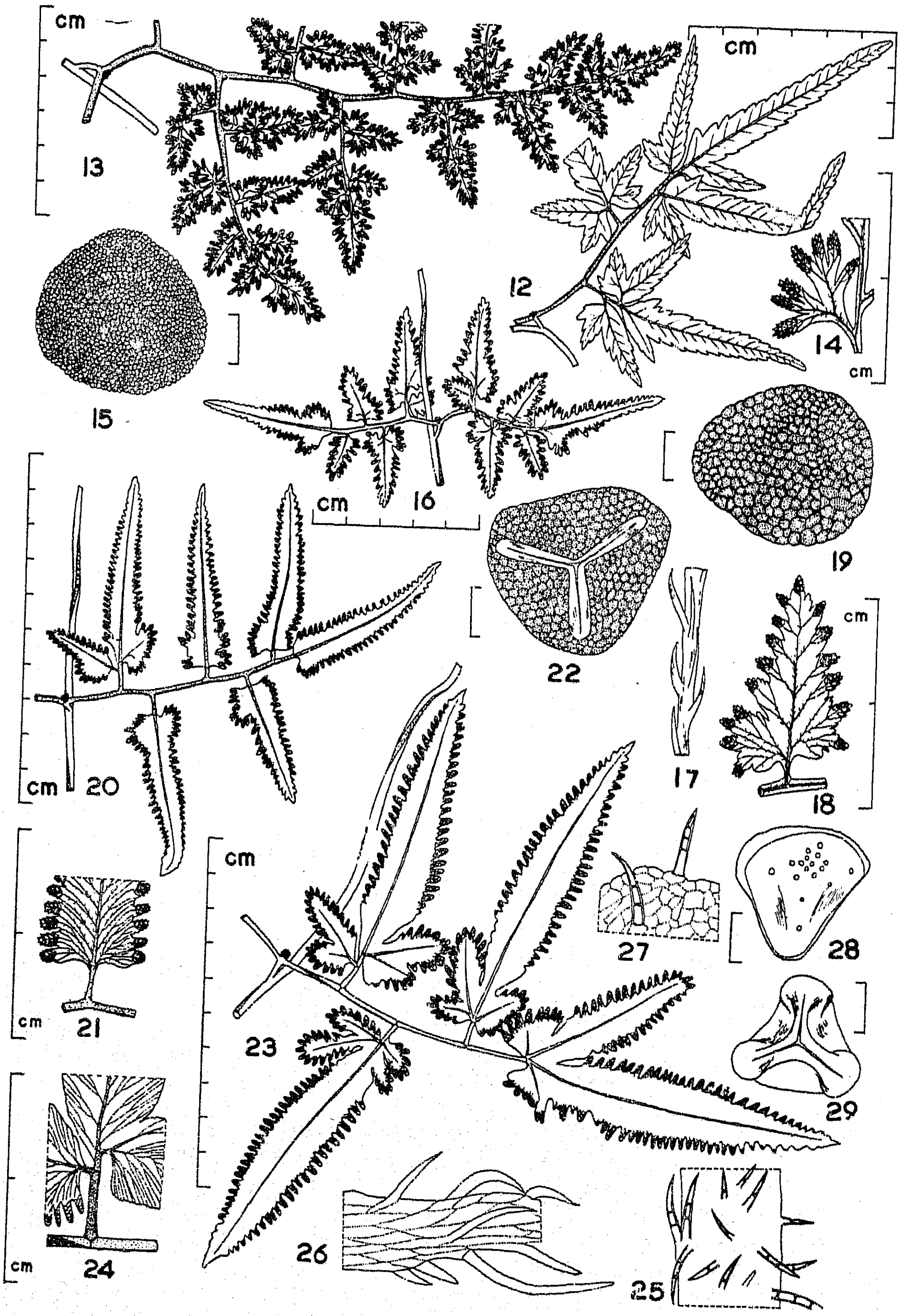
tribution: India (almost in all the states) Sri Lanka, Burma, S. China, Thailand-tnam, Malaya Peninsula, Malesian Islands, Australia (Queensland) and the Pacific nds.

Lygodium giganteum Tagawa and Iwatsuki in Acta Phytotax. Geobot 22(3): 97. . 1967; et Flora Thailand 3(2): 63. f. 4. 10–13. 1979.

'ype: Thailand, Chiang Mai, *Tagawa, Iwatsuki & Fukuoka* T-2362 (KYO).

izomes short-creeping. *Fronds* 3–4 m long, 30–40 cm broad, linear; *rachis* –2·5 mm across, dorsal surface puberulous; *pinnae* each half 15–20 cm long, -20 cm broad, deltoid, tripinnate; *primary rachis-branches* 4–7 cm long, distinct, like in rachis; dormant apex bearing 1–1·5 cm brown septate, pointed hairs; dormancy ercomes sometimes; *secondary rachis-branches* 7–15 cm long, stout, joints beyond ondary rachis jointed or articulated; pinnate or bipinnate *pinnules* 5–10 cm long, 5 cm broad, largest at the base becoming smaller upwards, all stalked, may be at apex sile, terminal simple or forked, obtuse, basal pinnules with further lobed or cleft e; stalks 5–15 mm long, articulated to the secondary rachis, every junction as a whole ollen and streaked giving 'Y' shaped blackish brown appearance; every junction ond this similar except the stalk of the ultimate pinnules where the apex is swollen ıb-shaped) or if stalk indistinct then the pinnule apex stalk may not be swollen but n forming 'Y' shaped joint directly on the secondary rachis; *ultimate pinnules* 3–8 cm g, basal ones distinctly stalked with one or two lateral lobes, lobes adnate, oblique, use, crenate, midvein distinctly hairy, veins hairy, 1–3 times forked, in between also a hairs present, these very short; texture subcoriaceous. *Sorophores* 2–5 mm long, :s present on and at the margin of the indusium; *spores ca* 62-62-62 μm pale and very ominent, surface smooth with a few tubercles (figures 23–29).

cimens examined: India: *Assam*, Mikir Hills, Umpanai, 16 June 1979, *Singh* 72899 AM). *Manipur*, *Sine lect.* 1699; Shugnu, 12 Sept. 1956, *D. B. Deb* 2666; Central trict, 17 Feb. 1978, *R. D. Dixit* 58967; *Nagaland*, Naga Hills, *S. K. Mukerjee* 3372. *oram*, Lushai Hills, 26 Nov. 1903, *J. E. Leslie* 165 (CAL). Burma: Moulmein, 1854 *lect.* 17 (Acc. No. 26734); Upper Burma, July 1892 *Abdul Huk s.n.* (Acc. No. 26733);



Figures 12–29. **12–15.** *L. japonicum* (*Panigrahi* 14862); **16–19.** *L. mearnsii* (*Singh* 77559); **20–22.** *L. salicifolium* (*Panigrahi* 16712); **23–29.** *L. giganteum* (*Deb* 2666); **12, 13, 20, 23.** parts of pinnae; **16.** pinna; **17, 26.** hair on midvein dorsal surface; **25.** hair on dorsal surface of secondary and tertiary rachis; **27.** hair on indusium; **15, 19, 22, 28, 29.** spores (scale = 25 μm).

bang near Luajuyo, 1186 m, 7 June 1915, *A. Rodger* 126; Rangoon, *Lord Dalhousie* (Acc. No. 26525-CAL). China: Yunnan, 1875, *D. J. Anderson s.n.* Acc. No. 26851 L).

tribution: India: (Assam, Manipur, Nagaland and Mizoram): Burma, Thailand, nà (Yunnan). A new record for India.

Lygodium japonicum (Thunb.) Sw. in J. Bot. (Schrader) 1800(2): 106. 1801; ldome, *op. cit.* 457; Alston and Holttum, *op. cit.*: 14; Holttum, *op. cit.*: 50. f. 8d-f: igrahi and Dixit *op. cit.*: 221. f. 3a-g.

)phioglossum japonicum Thunb. Fl. Jap. 328. 1784.

ype: Japan, Herb. *Thunberg s.n.* (UPS-microfiche spec. no. 25221 and 25222 CAL!)

zome long-creeping. *Fronds* 2–3 m long, 20–60 cm broad, *rachis ca* 2 mm across, sal surface pubescent; *pinnae* in sterile frond tripinnate and fertile frond quadripin-e to further decompound, ovate-deltoid; *primary rachis-branches* 6–8 mm long, bescent, dormant apex hairs *ca* 1 mm long, brown and pointed; *secondary rachis-nches* 10–30 cm long, in sterile frond pinnate and in fertile frond tripinnate to ther decompound, pubescent, none of the primary, secondary or tertiary rachis culate, ultimate pinnules auricled, ovate or deltoid–lanceolate, margin in sterile nd crenate and deeply cleft to the midvein in fertile; apex of the pinnule stalks not ollen; lamina pubescent; midvein and veins pubescent below; texture firm, blackish-wn when dried. *Sorophores* up to 7 mm long, 4–7 solitary sporangia, *spores c.* 96)6 × 96 μm, colliculate. (figures 12–15)

cimens examined: India: *Uttar Pradesh*, Kumaon, near Bunna, 1167 m, *Strachey l Winterbottom s.n.*; *Assam*, *Jenkins s.n.*; Kamrup District, Rangia, Athiabari 24 June 54, *A. S. Rao* 29070; *Meghalaya*, Khasi hills, Feb. 1851 *sine lect. s.n.* (Acc. No. 26806) L); *Arunachal Pradesh*, Subansiri district, Petepool I.B. to Zero, 10 Sept. 1959 *nigrahi* 19742; Tirap district, Nignu to Niosa, 29 Aug. 1958, Panigrahi 14862 (ASSAM). *galand*, Kohima, Dec. 1886 *Prain s.n.*; *Manipur*, Imphal 1000m, Feb. 1906, *Meebold* 0; Kangoi, Dec. 1907, *Meebold* 6888 *Mizoram*, Aizwal, 11 Jan. 1963, *D. B. Deb* 30646 L).

tribution: India (S. India, Assam, Meghalaya, Arunachal Pradesh, Nagaland, nipur and Mizoram); Sri Lanka, China, Japan, Korea, Malaya Peninsula, Malesian nds, Thailand-Vietnam, the Philippines and Australia (Queensland).

Lygodium longifolium (Willd.) Sw. in J. Bot. (Schrader) 1801(2): 305. 1803; ton and Holttum *op. cit.*: 19; Holttum, *op. cit.*: 59. f. 5a. 12.

Iydroglossum longifolium Willd. Abh. Kurf. Mainz. Ak. Wiss. Erfurt 2(4): 22. t. 2. 802.

ype: Malabar. Herb. *Willdenow s.n.* (B—microfiche no. 1410 spec. no. 19482 CAL!) *ygodium dichotomum* sensu Beddome, Ferns S. India t. 62. 1863; *non* (Cav.) Sw. 1806).

Rhizomes short-creeping. *Fronds* very long, sterile 30–45 cm broad, fertile 18–60 cm broad, juvenile fronds once or twice dichotomous each branch bearing a palmately lobed 4–5 subequal lobes, each pinnae cuneate to cordate at base; *rachis* 1·5–2·5 mm across, glabrous; *pinnae* each half 9–30 cm long, 7–40 cm broad, once or twice dichotomously branched, tripinnate, *primary rachis-branches* indistinct, dormant apex hairs 0·5–1·5 mm long, base of the hairs swollen, brown or black; *secondary rachis-branches* 5–10 cm long, pubescent, once forked in sterile and twice in fertile, ultimate lobes (sterile) forked and palmatisect, each lobe 2–3·5 cm broad, lanceolate, acuminate, crenate; veins twice or thrice forked, ending into crenation; lower surface with hairs scattered, upper surface also with scattered very long, septate, brown pointed hairs on the junctions with veins; (fertile) 1–2 cm broad, lanceolate, gradually acuminate, surface smooth or having hairs at the base or on sorophores both sides, veins once or twice forked, very prominent, texture coriaceous. *Sorophores* up to 4 mm long, 1 mm wide, upper surface with a few hyaline hairs; indusium not hairy; *spores* 133-133-133 × 137-137-137 μm, irregular reticulum with tubercles, pale brown (figures 40–44).

Specimens examined: India: *Meghalaya*, Khasi Hills, 1869, ex herb. *Jordon s.n.* (Acc. No. 26521) (CAL). Sri Lanka, *sine lect s.n.* no Acc. No. (CAL) Singapore, Chau Chu Kay, 1891, *sine lect.* 8057 (CAL). The specimen "Khasia Hills, year of collection 1869 *ex. herb. Jordon s.n.* locality doubtful, sheet from CAL" identified by Panigrahi and Dixit (1967) with *L. circinnatum* represents *L. longifolium.*

Beddome's (1863) illustration t. 62 is based on *Johnston s.n.* from Travancore (K), according to Alston and Holttum (1959).

Distribution: India (Meghalaya, Kerala); China (Hainan) Malaya Peninsula.

7. ***Lygodium mearnsii*** Copel. in Philip. J. Sci. 3 c: 37. 1908 et Fern Fl. Philip. 1: 35. 1958.

Type: Insula Batan, *Mearns s.n.* (Bur. Sc. 3136).

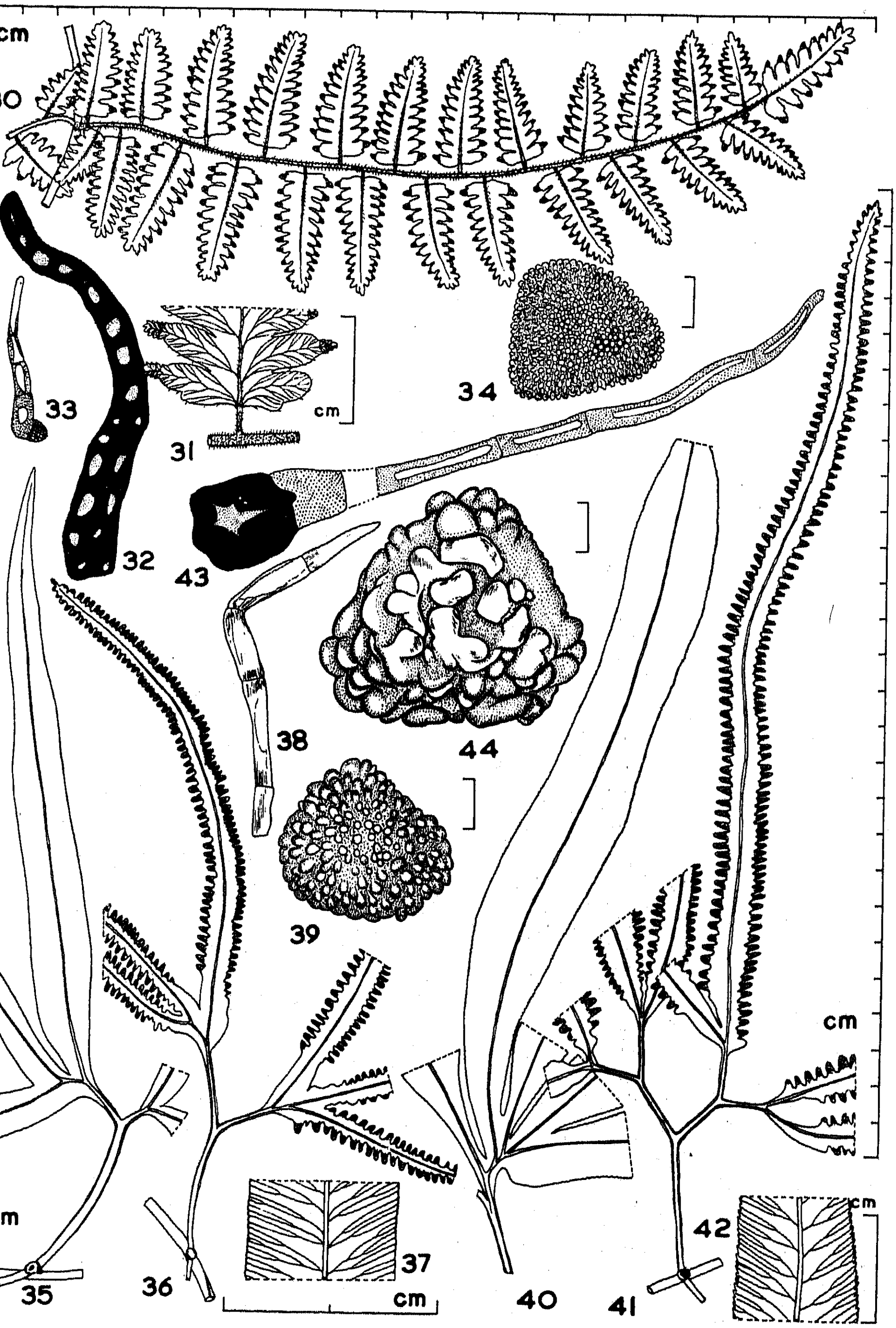
Lygodium japonicum sensu Holttum *op. cit.*: 50.*p.p.quoad. L. mearnsii* Copel. *pro. syn.*

Rhizomes short-creeping. *Fronds* 2–3 m long, tripinnate, *rachis ca* 1 mm across, pubescent on dorsal surface: *pinnae* 7–10 cm long, 6–8 cm broad, bipinnate, deltoid; *primary rachis-branches* c. 4 mm long, dormant apex hairs light brown, upto 8·5 mm long; *secondary rachis-branches* 3–7 cm long, pinnate, with basal pinnules or ultimate pinnules auricled; ultimate-pinnules deltoid-ovate to ovate-lanceolate, basal ones stalked and largest 3–5 cm long, 1·5–2·5 cm broad, terminal ones subsessile to sessile, auricled or cordate at base, acute; in sterile the margin crenate and in fertile fronds not deeply cut, costule, midveins and veins hairy; veins forked, hairs pale, drying pale green, texture herbaceous. *Sorophores* 1–3 mm long, 3–5 sporangia on each side, indusium margin fringed or hairy; *spores* 64-72 × 50-64 μm, pale green, surface irregular (figures 16–19).

Specimens examined: India: *Arunachal Pradesh*, Tirap District, Miao, *ca.* 350 m, 11 Aug. 1979, *Singh* 77559; *Lohit district, Deuning*, 19 *Sept.* 1969, *A. S. Rao* 47937 (ASSAM).

We have compared the Indian specimens with the Philippines (Babuyan Ist. *Herb. Bur. Sc.* 3916) available at CAL.

We have got a few specimens from Dehradun and Goa closely resembling *L. mearnsii* Copel.



Figures 30–44. 30–34. *L. polystachyum* (*Kurz* 778); **35–39.** *L. circinnatum* (*King's collector s.n.*) **40–44.** *L. longifolium* (40, 42-*Jerdon s.n.* and 41, 43, 44-*sine lect* 8057); **30, 35, 36, 40, 41.** parts of pinnae; **40, 41,** portion of pinnae showing edges; **32, 33, 38, 43.** hairs of dormant apices; **34, 39, 44.** spores (scales = 25 μm).

Distribution: India: (Arunachal Pradesh); the Philippines (Baten, Babuyan Islands). This is a new record for India.

L. mearnsii Copel. was merged with *L. japonicum* (Thunb.) Sw. by Holttum (1959); the distinguishing characters delineated by Copeland and as set out in the key above, appear reasonable. Besides, differences in the size of the spores viz 80–100 μm in *L. flexuosum vis-a-vis* 64-72 × 50-64 μm in *L. mearnsii* are also distinctive. *L. mearnsii* appears to be intermediate between *L. flexuosum* and *L. japonicum*, differing from the former in having an elongate primary rachis.

8. *Lygodium microphyllum* (Cav.) R. Br. Prod. Fl. Nov. Hollandae: 162. 1810; Beddome, *op. cit.*: 455 t. 282; Alston and Holttum *op. cit.*: 12; Holttum *op. cit.* 47. f. 5e-f. 6, 7; Panigrahi and Dixit, *op. cit.*: 224. f. 4.

Ugena microphylla Cav. Ic. Descr. Pl. 6(2): 76. t. 595. f. 2. 1801.

Type: Luzon, *Nee s.n.* (MA-vide Holttum *l.c.*)
Lygodium scandens sensu Sw. in Journ. Bot. (Schrad.) 1800(2): 106. 1801. *p.p. excl.* typo, *non* (L.) Sw.; Beddome, Ferns S. India t. 61. 1863.
Ophioglossum filiforme Roxb. ex Griffith in Calc. Journ. Nat. Hist. 4: 476. t. 26. f. 3. 1844;
Iconotype: Roxb. Icon. No. 748 (CAL! K) (see Sealy, Kew Bull. 11: 364. 1956).

Rhizome long-creeping. *Fronds* reaching to a length of 5–7 m, 15–45 cm broad, tripinnate; *rachis c.* 2 mm across, surfaces glabrous; *pinnae* each half 12–20 cm long, 4–8 cm broad, ovate-oblong, pinnate; *primary rachis-branches* 3–5 mm long, dormant apex bearing 0·5–1·5 mm long, brown, septate hairs; *secondary rachis-branches* glabrous; ultimate pinnules 1–4·5 cm long, 1–2 cm broad, all stalked (terminal ones also), stalk 2–4 mm long, apex of the stalk swollen *i.e.* pinnule lamina articulated with stalk, when dried these pinnules fall off from this junction leaving stalk attached to the secondary rachis; ovate-oblong, base cordate or auricled, entire or just crenulate, obtuse, surfaces smooth; veins many times forked, prominent, ending into the margin, sterile pinnules comparatively larger than the fertile. *Sorophores* up to 3 mm long, 4–5 sporangia on each side, *spores* 62-62-62 × 65-65-65 μm, surface with reticulum (figures 5–8).

Specimens examined: India: Kerala, Valiaparathode, 800 m, 20 Apr. 1980, *Vohra & Ghosh* 56265; *Tamilnadu*, Yerkaud, 1857 m, 13 Sept. 1958, *Subramanyan* 6879; *Assam*, N. Lakhimpur, 14 Mar. 1962, *Panigrahi* 27859 (CAL); Jorhet, 13 Sept. 1978, *Singh* 72761; Cacher district, Joharband R.F. 24 Aug. 1978, *Singh* 72733 (ASSAM); *Meghalaya*, Garo Hills, 50 m, Feb. 1903, *Morten s.n.* (CAL); Baghmara, 12 Dec. 1960, *Panigrahi* 22541 (ASSAM). *Arunachal Pradesh*, base Duphla Hills, 1874, *Lister s.n.* (CAL); Tirap district, Nampong, *c.* 123 m, 12 Oct. 1959, *R. S. Rao* 20036 (ASSAM); *South Andaman*, Wandur, sea level, 3 July 1974, *Balakrishnan* 1669 (CAL).

Distribution: India (S. India, North Eastern India) Sri Lanka, Africa, Melanesia, Malaysia, Hong Kong, Australia (Queensland).

9. *Lygodium polystachyum* Wall. ex Moore, Gard. Chron. 671. 1859; Beddome, *op. cit.*: 458 t. 284; Alston and Holttum *op. cit.*: 11; Holttum, *op. cit.*: 46–47, f. 5c. 8a–c.

Type: Malaya, Penang, *Wallich* 177 K-W, Microfiche CAL! isotype-CAL!

Rhizome short creeping. *Fronds* long, 45–90 cm broad; *rachis ca* 2 mm across, terete, not winged, pubescent to hairy throughout; *pinnae* each-half 22–45 cm long, 7–13 cm broad, oblong or lanceolate-oblong, quadripinnatifid, acute; *primary rachis-branches* indistinct, terete, densely hairy, dormant apex bearing 1–1·5 mm long septate, blackish brown, pointed hairs, mature hairs thick either with multiseptate swollen base or to a certain length, *secondary rachis-branches* 20–35 cm long, stout but brittle, terete, densely hairy throughout, bipinnatifid; *pinnules* 3–7 cm long, 1·5–2 cm broad, alternate, basal 2–3 pairs reduced, stalked, stalk *ca* 5 mm long, densely hairy, swollen at the junction with lamina; ovate-lanceolate, base cordate or auricled, truncate, deeply lobed, obtuse, deciduous; midvein hairy on both surfaces, prominent; lobes 3–5 mm broad, entire, veins once or twice dichotomously branched, hairy on both surfaces; in fertile fronds these lobes bear terminal sorophores, sorophore region when mature conspicuously contracted. *Sorophores* 5–7 mm long, *ca* 2 mm broad; indusium hairy on surface and at margin; *Spores* 74-74-74 × 76-76-76 μm, pale brown, finely verrucose (figures 30–34).

Specimens examined: No representation from India in CAL. Burma: Martaban, *S. Kurz* 778; Pegu, *S. Kurz* 7768?, Tenasserim, Bonachoung, April 1911, *Meebold* 15268 (CAL).

Distribution: India (Assam); Burma, China, Thailand, Cambodia, Laos, Vietnam, Malaysia.

We have seen all the Herbarium sheets of the genus *Lygodium* in CAL and ASSAM but could not spot even a single specimen from Assam or any other Indian localities. Our record of the occurrence of the species in India is based on Holttum (1959).

10. *Lygodium salicifolium* Presl, Suppl. Tent. Pterid. 102. 1845. *p.p. excl. pl. Wallich p.p.* and *syn. L. semibipinnatum* Wall.; Alston and Holttum, *op. cit.*: 14: Holttum, *op. cit.*: 51. f. 6. 10. 13a–b.

LT.: Singapore, *Cuming* 365 (PR, selected by Holttum 1959 as type); isolectotype-W, K.

Lygodium flexuosum sensu Bedd. Handb. Ferns Brit. India 457. 1883 *p.p. include L. longifolium* Wall. *nom. nud. tantum pro syn.*

Lygodium longifolium Wall. Num. list. p. 7. no. 175. 3 and p. 63 no. 175. 3. 6. 1829. *nom. nud.*

Rhizome short erect. *Fronds* 30–450 cm long, 25–70 cm broad; *rachis ca* 2 mm across, stout, dorsal surface pubescent; *pinnae* 10–35 cm long, 11–30 cm broad, tripinnate, sometimes quadripinnate, ovate or deltoid, *primary rachis-branches* indistinct to 2 mm long, dorsal surface pubescent, dormant apex bearing 1–1·5 mm long, septate brown hairs; *secondary rachis-branches* 7–25 cm long, winged narrowly, pinnate usually, sometimes bipinnate; *pinnules* 4–13 cm long, 1·5–6 cm broad, (more than 2 cm broad when basal pinnule further pinnate* usually 3–5 pairs, up to 7 pairs alternate all stalked; stalks 2–8 mm long, apex swollen at the junction with lamina, all pinnules ± equal in size, base wide cuneate to cordate or auricled, lanceolate to deltoid-lanceolate, obtuse, terminal

* Wall. Cat. 175.3. (K-W microfiche CAL!) shows up fronds with secondary pinnae bipinnate and Presl described *L. salicifolium* as "*infimae saepe trifoliolato-pinnatae*". Yet, Holttum (1959) delineates *L. salicifolium* as having the secondary pinnae only lobed at base.

pinnae similar to lateral, in some cases the basal pairs of the pinnules further pinnate then all of them nearly equal in size: pinnule-lamina articulated to stalks, in dried and older specimens the leaflets break-off from this point, crenate to sub-entire; midveins hairy, veins once or twice forked, prominent, hairy; texture firm. *Sorophores* up to 5 mm long, a few hairs on dorsal surface; *spores* 82-82-82 × 84-84-84 μm, colliculate or finely verrucose (figures 20–22).

Representative specimens examined: India: Goa, Maduka bungla jungle, 22 Nov. 1962, *R. S. Rao* 85034; *Kerala*, Quilon, *Wight* 260; Vazhaohal, Chalakudy, 400 m, 11 Sept. 1974, *Nair and Ghosh* 52074. *Sikkim*, 1862, *Anderson* 1408; *Assam*, C. N. Hills, Ralsosile, 22 Oct. 1852, *Godfrey* 255; Cachar, 1890, *Prazer* s.n. (CAL); Loharband R. F. 24 Aug. 1978. *Singh* 72733 (ASSAM). Haflong, 850 m, 5 Aug. 1908, *Craib* 165. *Meghalaya*, Pandua (Border K and J Hills) 11 June, 1850, *J. D. Hook and T. T.* 427; Khasi Hills, 2000 m, below Cherra, 12, 1880, *Fraser s.n.*; Garo Hills, *Srinivasan* 1951. *Arunachal Pradesh*: Subansiri District, *sine lect* 93; (CAL), Tirap District Khela to Changlang, *ca* 600 m, 16 Mar. 1958, *G. G. K. Murty* 12987; Niusa to Wanu, 3 Sept. 1958, *Panigrahi* 16712; Deomali, 188–166 m, 19 Oct. 1959, *R. S. Rao* 20305 (ASSAM). *Nagaland*, Naga Hills, *ca.* 300 m, 2 Feb. 1882, *Collett* 37; *Manipur*, Imphal, 28 Jan. 1960, *Mukerjee* 5244; *Mizoram*, Aijwal, 13 Jan. 1963, *Deb* 30645; *Tripura* (Tipperah) Abhoynagas, West of Agartala, 200–300 m, 27 Dec. 1914, *Debbarman* 403. *Andaman islands, sine lect* 10793; Between Ograbary & Partmouat, 28 July 1894, *King's collector s.n.*; Herbertabad, sea level, 14 Dec. 1973, *Balakrishnan* 691 (CAL).

Some of the specimens included under *L. flexuosum* (L.) Sw. by Panigrahi and Dixit are here identified with *L. salicifolium.*

Distribution: India (Kerala, Bihar, Assam, Meghalaya, Arunachal Pradesh, Tripura, Andaman and Nicobar Islands) Burma, China (Yunnan) Thailand Vietnam, Taiwan, Malaya Peninsula to Malesia to New Guinea.

Alston and Holttum (1959: 14) remarked that "this species appears to intergrade in some measure with *L. floxuosum* (L.) Sw., and in Holttum, Ferns of Malaya (p. 57) the two are united, but typical habitat of the two species are quite distinct. It is possible that hybridization occurs, but no experimental investigation has been made. *L. floxuosum* has the wider distribution, probably *L. salicifolium* is confined to moister habitats or regions with a shorter dry season".

6. Ecology

Lygodiums are true climbing ferns. The plants climb to a height of 1 to 10 m, *L. microphyllum* and *L. salicifolium* climbing much higher than the others. The rachis which is tough and plastic can twist and twine around nearby bushes or trees and on bamboo grooves, and in open situations in secondary forests mostly on the edges of forests. Sometimes growth is so vigorous that the plants cover the entire surface of small trees or big bushes thus forming thickets. In many cases the newly formed rachises twine around the old living and dried-fronds giving a rope like appearance. *L. japonicum* grows from plains to higher altitudes in sub-tropical forests, a few species love comparatively wet climate. In contrast, *L. flexuosum* (L.) Sw. is almost pantropical and subtropical and occurs also in dry deciduous forests of India.

Cytology: Panigrahi and Dixit (1967) have tabulated the available cytological information on the genus and discussed the impact of such studies on the origin of the haploid numbers $n = 28$, 29 and 30 and the resulting implications of such findings on the phylogenetic affinities of the genus *Lygodium*.

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Pharmacognostic studies on 'Sappan' (*Caesalpinia sappan* Linn.) and its market samples*

SHANTA MEHROTRA and H P SHARMA
Pharmacognosy Laboratory, National Botanical Research Institute, Lucknow 226 001, India

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Abstract. 'Sappan' is considered a valuable astringent, alterative tonic, emmenagogue, blood purifier and anticoagulant. It strengthens the bones and teeth and is also used in boils and eruptions. The drug is ascribed to the heartwood of *Caesalpinia sappan*. Accordingly, a detailed pharmacognosy of *Caesalpinia sappan* was carried out and eight market samples of 'Sappan' procured from various parts of the country were evaluated with reference to the genuine drug. The present studies revealed that out of all the market samples studied only those from Bombay (B-II), Rishikesh and Hardwar resembled the genuine drug and were therefore identified as genuine *C. sappan*. The remaining samples were identified as different *Pterocarpus* species, *Gluta travancorea* Bedd. and *Toona ciliata* M. J. Roem.

Keywords. *Caesalpinia sappan*; heart wood; Caesalpiniaceae; pharmacognosy.

1. Introduction

'Sappan', which is ascribed to *Caesalpinia sappan* Linn. (family–caesalpiniaceae), is regarded as one of the important drugs of indigenous systems of medicine. It has also been referred to as 'Raktasar', 'Suranja', 'Pattaranjaka' in Ayurvedic texts and is considered as one of the 'Chandan' group of drugs (Dwivedi 1949; Gor 1935; Saligram 1953).

The heartwood, which forms the actual drug, is considered a valuable astringent, alterative tonic, emmenagogue, blood purifier, anticoagulent and is said to be useful in diarrhoea, specially of the children (Ainslies 1826; Anonymous 1950; Bhandari 1949; Chopra *et al* 1956; Dey 1896; Khory 1887; Waring 1868; Watt 1962). It is claimed that a decoction of the drug, cleans the surface of the body, improves the complexion, cures skin diseases and strengthens the bones and teeth (Gupta 1950; Playfair 1833; Sharma 1978). It is also useful in leprosy, insanity, epilepsy and urinary concretions as claimed by Kirtikar and Basu (1933) and Rao (1914). Further, according to Dwivedi (1949) it is applied to wounds and ulcers in the form of a paste.

Nadkarni (1954) reported that 'Gulal'—made from arrow root and red colouring of Sappan wood—is used in 'Otorrhoea' by being blown into the ear. In combination with other drugs it is also used in dental treatment (Charak 1945). Besides, Sappan forms an ingredient of several proprietary formulations used for leucorrhoea as well.

As is well known a lot of adulteration/substitution in the marketed crude drug samples goes undetected. It was, therefore, considered worthwhile to study the commercial materials of 'Sappan' procured from different markets of the country so as to test the authenticity of the different samples.

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2. Previous work

Brazilin was isolated from *C. sappan* wood by Kisser (1823) and essential oils of its leaves were studied by Romburg (1925, 1927). Hayashi (1950) identified the red dyes obtained from the Sappan wood, whereas gallotannin distribution, biogenesis and physiological action of *Caesalpinia* species and certain other genera were investigated by Paris (1967). Hikino *et al* (1977) studied the anti-inflammatory principles of *Caesalpinia sappan* and *Haematoxylon* sp. woods and Nigam *et al* (1978) isolated α-amyrin and β-sitosterol from Sappan wood. However, no detailed work on pharmacognosy of Sappan could be traced and hence the present studies.

3. Material and methods

Fresh material of *C. sappan* was collected from the Indian Botanic Gardens, Sibpur, Calcutta, and eight market samples of the drug 'Sappan' were procured from the drug markets of Calcutta, Bombay, Lucknow, Rishikesh and Hardwar for authentication.

Wood microtome sections (TS, RLS and TLS) of the different samples were employed for detailed anatomical studies and matched with authentic *C. sappan* wood sections at the Forest Research Institute, Dehra Dun.

For preliminary phytochemical studies a known quantity of dried powder was extracted in a Soxhlet apparatus with hexane, benzene, chloroform, alcohol and water successively and the extracts tested for different constituents. Total ash, acid insoluble ash, alcohol and water soluble extractive percentages were calculated according to IP procedures (Anonymous 1966). Tannin percentages (calculated as gallo-tannins and crude tannins) were determined according to AOAC method (Anonymous 1965) and fluorescence characters analysed according to the methods described by Chase and Pratt (1949) and Kokoski *et al* (1958).

4. Studies on the genuine sample

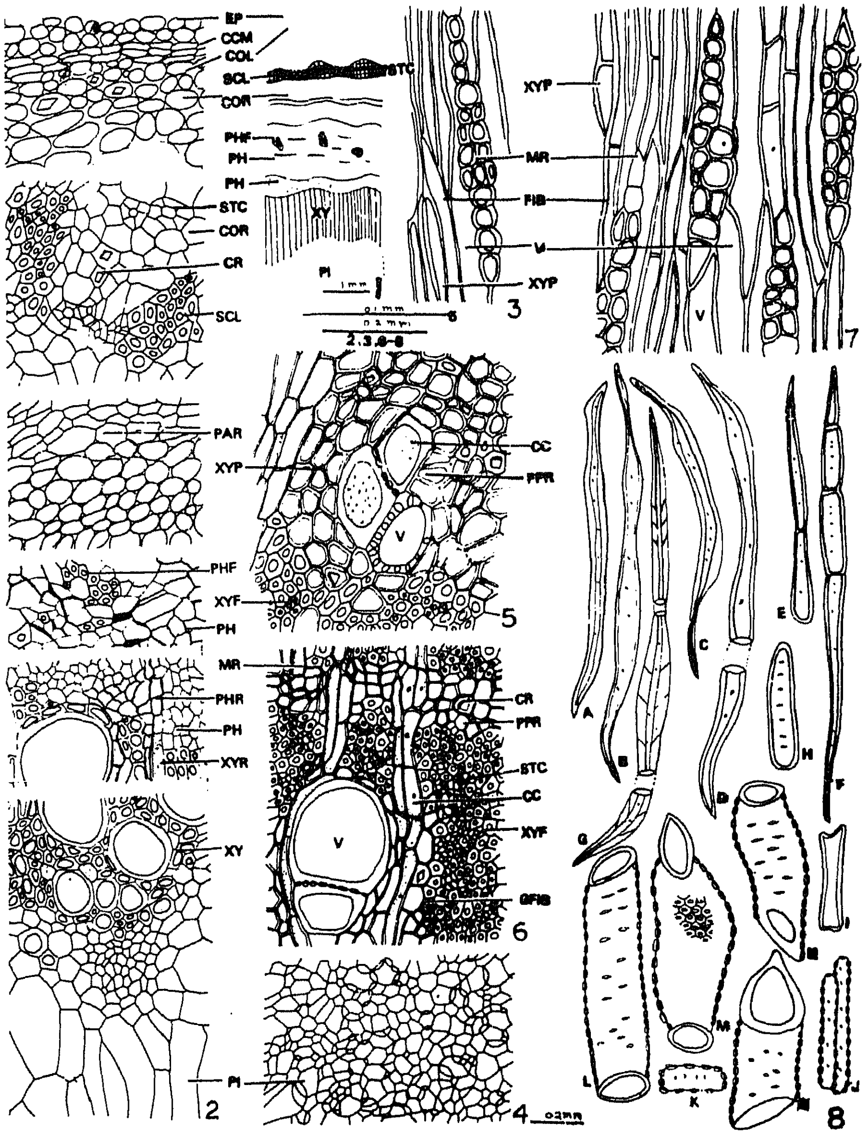
4.1 *Macroscopic characters*

C. sappan is a 6–9 m tall tree attaining a diameter of 20–38 cm. Its leaves are sub-sessile and the flowers yellow. It occurs widely in Southern parts of the country and Bengal and extends up to Sri Lanka, Burma and Malaya.

The sapwood is easily distinguishable from heartwood since the former is buff-coloured whereas the heartwood is orange yellow, heavy (sp. gr. 1.03), fairly straight-grained and diffuse porous. The growth rings are demarcated by fine, interrupted lines of parenchymatous cells. The wood has a bitter taste, is odourless and shows a hard fracture.

4.2 *Histology*

4.2*a* *Stem*: A TS of mature stem appears almost circular in outline. The epidermal cells are oval in shape, and have a thick cuticle on the outside (figures 1 and 2). This is followed by a parenchymatous, many-layered cortex filled with yellow contents and interspersed in between are fibres and stone cells. Prismatic crystals of calcium oxalate are



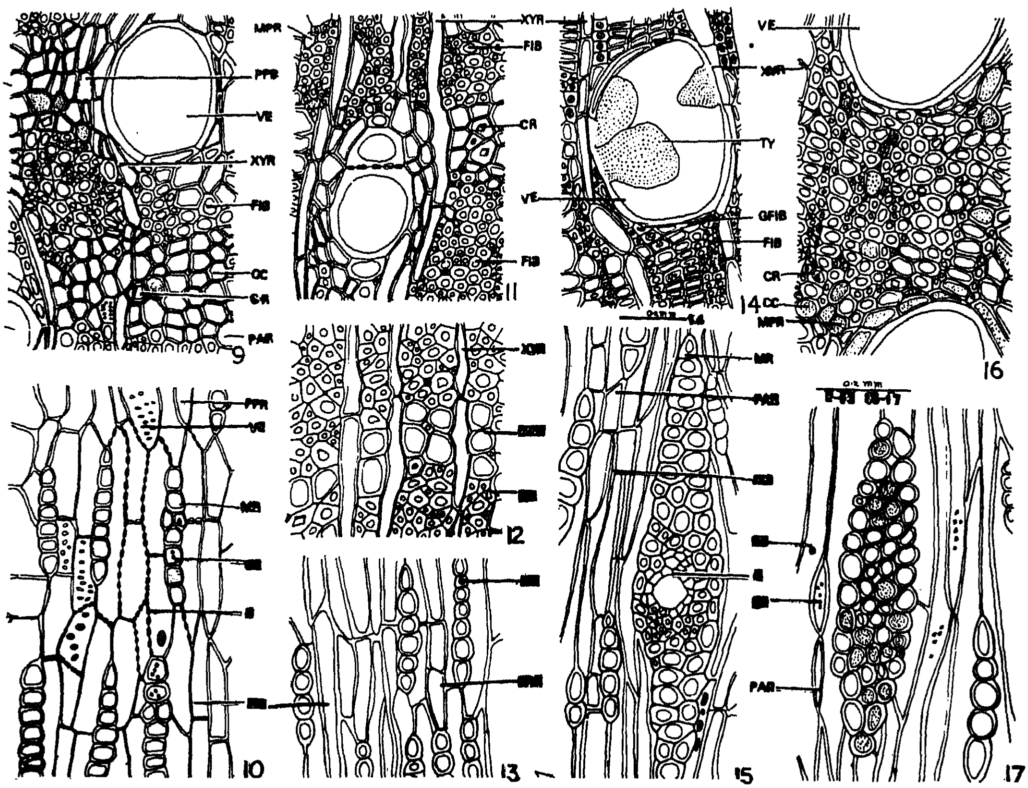
Figures 1–8. **1.** TS young wood (diagrammatic). **2.** A portion of above showing details. **3.** TLS of young wood showing medullary rays and xylem. **4.** TS of young wood passing through pithregion. **5, 6.** Portions of the TS of mature wood showing details. **7.** TLS of mature wood showing details. **8.** Isolated elements of wood. **A–D.** fibres. **E–F.** septate fibres. **G.** gelatinous fibres. **H–K.** parenchymatous cells. **L–O.** different shapes of vessels. Explanation for abbreviations given in p. 150.

also present in the cortical cells. The cork cambium arises in the sub-epidermal region. The phloem consists of sieve-tubes, companion cells, phloem parenchyma and irregularly-scattered groups of fibres. In some places primary phloem is crushed in the form of bands. The xylem is well-developed and composed of tracheids, vessels and xylem parenchyma. The pith consists of variously shaped parenchymatous cells with simple pits on their walls (figure 4).

4.2*b* *Heartwood* The heartwood consists mostly of small xylem vessels. A few to moderately numerous, medium-sized vessels which are visible to the naked eye, are also present. The vessel density is 5–9 per mm^2 and their size 25–525 × 10–100 μm. They are oval to orbicular in TS and are either solitary or arranged in radial rows in multiples of 2 or 3. The pits on radial walls are simple and alternate with lenticular pit pores (figures 8L–O). Similar pits were observed in tracheids as well. Tyloses are absent but vessels are filled with yellowish white deposits.

The xylem parenchyma is paratracheal, paratracheal zonate or metatracheal type (figures 5, 6). The paratracheal parenchyma forms a narrow, 1–3 seriate sheath, frequently interrupted by rays and fibres. In TS such parenchymatous cells appear hexagonal, have simple pits on the walls and are filled with dark brown contents. In paratracheal zonate type the concentric bands are separated by wider bands of fibres (figure 6). The metatracheal type is, however, 1–3 seriate, elongated, sparse and restricted to the zones of fibres. Solitary prismatic crystals of calcium oxalate are found in paratracheal and paratracheal zonate parenchyma but are absent from metatracheal ones.

Fibres are long, septate, libriform to non-libriform and hexagonal in TS (figures 5, 6).



Figures 9–17. 9–10. TS and TLS of wood of Bombay-I sample showing details. **11–12.** TS of wood of Lucknow-II sample showing details. **13.** TLS of same showing details. **14–15.** TS and TLS of wood of Calcutta-I sample showing details. **16–17.** TS and TLS of wood of Calcutta-II sample showing details.

Explanation for abbreviations given in p. 150

The septate fibres are two- to three-celled and have tapering to rounded ends (figures 8E, F). Interfibre pits are both simple and bordered (figures 8A, D). Long, irregular, gelatinous fibres were also observed occurring between other types (figure 8G). These are filled with yellowish brown pigments.

The rays are fine and discernible as short, thin and slightly darker streaks in the wood even to the naked eye. Their frequency is 6–8 per mm in the young and up to 14 in the mature wood. The rays are heterogenous, usually uniseriate, 2–38 cells high and 1–4 cells wide in the young stem (figure 3). In the older stems these are, however, biseriate. The upright cells in RLS are rounded while the procumbent ones are more or less rectangular in outline (figure 7).

5. Studies on market samples

5.1 *Bombay I*

In a TS the heartwood shows distinct to indistinct growth rings unlike the genuine sample and has medium to large-sized vessels. The narrower ones of these occur towards the outer side of the growth ring progressively increasing in width inwards. The vessels are usually solitary but sometimes occur in radial rows of 2–4 with a density of 1–13 per mm^2. These are usually plugged with golden brown to white deposits and the successive ones are separated by parenchymatous cells. The vessels bear simple pores with prominent collars on the end walls but the lateral walls have oval to orbicular bordered pits arranged in an alternate manner. The vessel segments are short (figure 20), truncate or rarely short tailed.

The xylem parenchyma may be paratracheal, paratracheal zonate or metatracheal type as in the genuine sample (figure 9) but paratracheal parenchyma, in this case, forms a narrow 1- to several seriate sheath frequently interrupted by rays and less frequently by fibres. In the paratracheal zonate parenchyma the concentric bands are separated by wider bands of fibrous tissue. Prismatic crystals of calcium oxalate are found in the marginal cells of paratracheal zonate parenchyma.

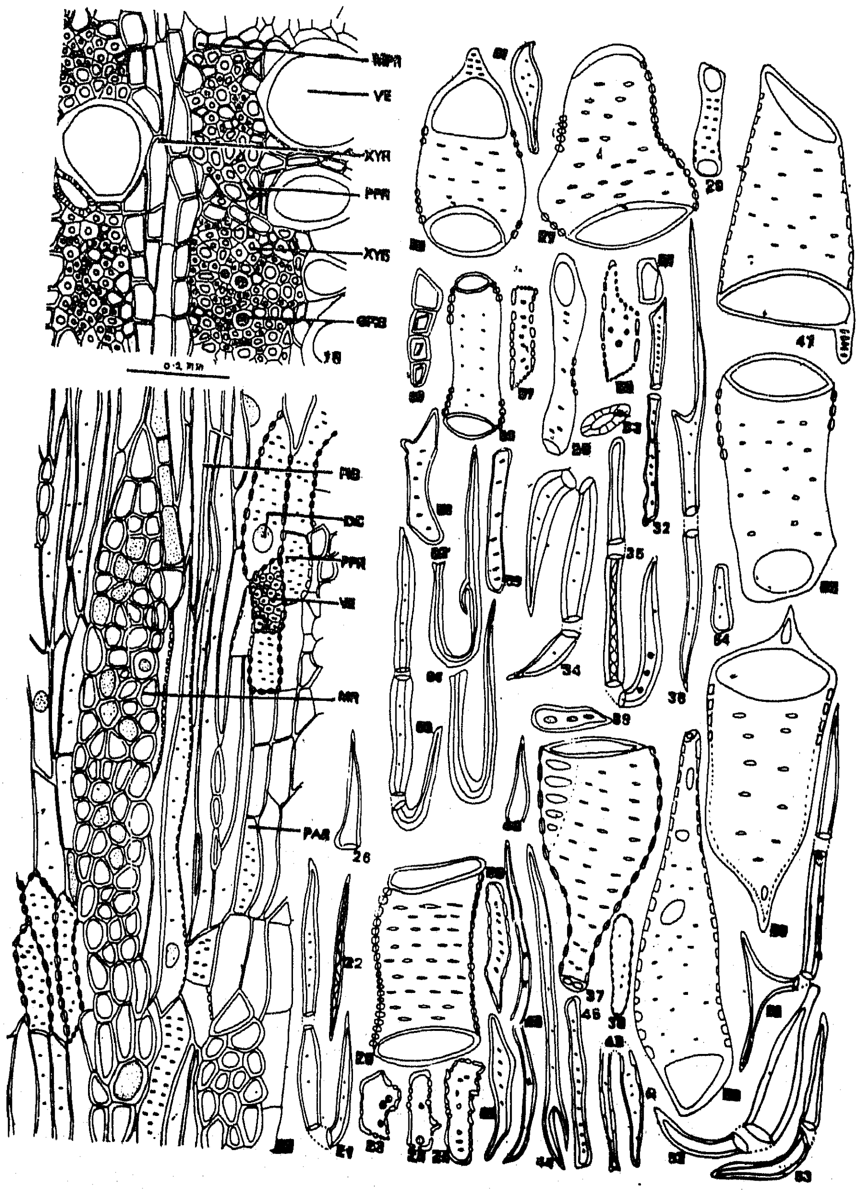
Xylem fibres are non-septate, non-libriform to semi-libriform and angled in TS (figure 9), mostly continuous, wavy and often filled with yellow brown contents. Sometimes these occur in anastomosing bands alternate with narrow bands of parenchyma. Interfibre pits are simple and confined mostly to the radial walls (figures 21, 22, 26). Occasionally, substitute fibres are also present in the zonate parenchyma.

Ripple marks of medullary rays are visible under a magnifying lens. Rays are extremely fine, close (9–13 per mm) separated by 1–4 rows of parenchyma, homogenous 4–10 cells high and 1-sometimes 2-cells wide (figure 10). Brown gummy infiltration is absent in the ray cells.

In isolated preparations the sample shows vessels (figure 20), different types of fibres (figures 21, 22, 26) and parenchymatous cells (figures 23–25).

5.2 *Lucknow I*

Vessels are large to medium sized, varying from 290 to 16800 μm. Like the previous sample the narrower vessels are arranged near the periphery of the growth rings and the broader ones are successively inwards, again separated mostly by bands of paratracheal



Figures 18–64. 18–19. TS and TLS of wood of Lucknow-I showing details. **20–26.** Isolated elements of wood of Bombay-I sample. **20.** vessel. **21, 22, 26.** fibres. **23–25.** parenchymatous cells. **27–36.** Isolated elements of wood of Lucknow-II samples. **27–29.** different shapes of vessels. **30.** parenchymatous cells. **31.** parenchymatous cells containing crystals of calcium oxalate. **32, 34–36,** fibres. **33.** stone cell. **37–46.** Isolated elements of wood of Calcutta-I sample. **37.** vessel. **38–41.** parenchymatous cells. **42–46.** fibres. **47–54.** Isolated elements of wood of Calcutta-II sample. **47–50.** different shapes of vessels. **51–53.** fibres. **54.** parenchymatous cell. **55–64.** Isolated elements of wood of Lucknow-I sample. **55–56.** different shapes of vessels. **57.** parenchymatous cells. **58–59.** tracheids. **60.** prismatic crystals of calcium oxalate. **61–64.** fibres.

Explanation for abbreviations given in p. 150.

parenchyma (figure 18). The vessels are open but are sometimes filled with reddish brown pigments. The vertically contiguous vessels are connected through oval to orbicular pits somewhat similar to the previous sample.

Arrangement of parenchymatous cells is similar to that of the previous sample except that the paratracheal parenchyma, in this case, is mostly uniseriate and the paratracheal zonate parenchyma 2–4 layered. The metatracheal parenchyma is distributed sparsely in the fibrous zone. In some places the metatracheal zonate parenchyma forms a thin plate in between the fibres. All the parenchymatous cells are filled with starch and dark reddish brown contents (figure 18).

Substitute fibres are also occasionally present and have prismatic crystals of calcium oxalate (figure 60). Fibres are libriform to non-libriform, angled (in TS) and non-septate. Inter fibre pits are simple with short oblique lenticular orifices. Fibres are 400–1000 μm $\times$ 2–25 μm in size and their lumen is frequently occluded with golden yellow deposits (figures 61–64). Gelatinous fibres were also observed (figure 18).

The medullary rays are very fine, indiscernible to the naked eye, close (7–9 per mm) with 1–4 (mostly 2 or 3) rows of parenchyma in between and are 4–20 cells high and 1–4 cells wide (figure 19).

In macerated preparations different shapes of vessels (figures 55, 56), parenchymatous cells (figure 57) and tracheids were observed (figures 58, 59).

5.3 *Lucknow II*

The wood in this sample is storied and the vessels solitary, occasionally in radial rows of up to 10 vessels with an average density of 4–14 per mm^2. Vessel segments are short, truncate or abruptly tailed (figures 27–29) and the lumen is filled with reddish brown deposits. Arrangement of parenchyma is more or less similar to previous samples (figures 11, 12). Paratracheal parenchyma is, however, 1–2 seriate and peripherally flattened while the paratracheal zonate type is oval to rounded with a diameter of 30–40 μm. In both, paratracheal zonate and metatracheal parenchyma, crystals are abundant (figures 11, 30, 31).

The fibres are non-septate, libriform to semi-libriform fine and appear rounded in a TS (figure 12). These are arranged in concentric bands traversed irregularly by narrow bands of parenchyma. Non-septate fibres are mostly gelatinous, and 500–1420 μm $\times$ 2–20 μm in size. Some of these are extremely thick and the inter fibre pits are simple (figures 32, 34–36). Stone cells were also observed in macerations (figure 33).

The rays (figure 13) are very fine, 8–10 per mm (TLS), storied, homogenous, 4–10 cells high and 1–2 cells wide and pitted. Ripple marks are present but are indiscernible to the naked eye.

5.4 *Calcutta I*

The growth rings are indistinct to distinct; vessels 300–700 μm long, medium thick-walled, truncate or abruptly attenuate, solitary, less commonly in radial rows of 2–9 with a density of 1–16 per mm^2. The largest vessels are 255–300 μm in diameter; perforations simple, pits leading to contiguous vessels large, oval or angular with broad

border and linear lenticular orifices (figure 37). Vessels are occluded with reddish brown contents but occasionally with solitary, spherical or elliptical bodies resembling aggregate crystals of calcium oxalate. Tyloses are also present (figure 14).

All three types—paratracheal, paratracheal zonate and metatracheal parenchyma are present. Paratracheal parenchyma is sparse and narrow, 1- to several seriate and frequently interrupted by medullary rays or less frequently, by fibres. Metatracheal parenchyma is extremely sparse. In isolated preparations the parenchymatous cells are variously shaped (figures 38–41).

The fibres are non-libriform to semi-libriform, fine, aligned in radial rows (figures 15, 42–46) which are interrupted by the tangential bands of parenchyma. Gelatinous fibres are also present (figure 14). The non-septate ones are 485–590 μm long and 20–25 μm in diameter, have simple and bordered pits and contain brown deposits.

The medullary rays are fine, close and indistinguishable with the naked eye. They are heterogenous, fusiform, and the largest ones are 1–4 cells wide and 10–25 cells high. Unlike other samples gum canals are conspicuously present here (figure 15). These are horizontally arranged, 0–2 per mm^2 lined by secretory epithelial cells (figure 15) and are 30–60 μm in diameter.

5.5 *Calcutta II*

The wood in this sample is semi-ring porous to ring porous, and growth rings are distinct. The vessels are 4–10 per mm^2 with simple, nearly horizontal to oblique perforations. Vessel segments are short (190–485 μm), truncate with abrupt endings or attenuate and tailed like the previous sample (figures 47–50). Tyloses are also present.

The parenchyma is usually indistinct and the metatracheal type abundant, diffuse or contiguous and several celled. Reddish brown infiltration and crystals are quite common (figure 16).

The fibres are non-septate and non-libriform, 400–1750 μm long, 34–38 μm in diameter and 2–4 μm in thickness (figures 51–54).

Rays are visible with the naked eye, narrow, 4–8 per mm, 1–7, mostly 3–5 seriate (figure 17), lenticular in TS and heterogenous. The largest ones are 120–135 μm wide and 20–25 μm high. Crystals are occasional and solitary. Lysigenous, tangentially elongated gum canals are occasionally present which are filled with reddish brown gum deposits.

6. Study of powder

The behaviour of drug powders of the various samples treated with different chemical reagents and their fluorescence characters were studied and the results are presented in tables 1 and 2 respectively.

7. Phytochemical studies

Air-dried material was used for carrying out quantitative determination of ash values, alcohol and water extractives (IP method), tannin, and successive extractives—hexane, benzene, chloroform, alcohol and water. The results are summarised in histograms.

Powder +	Young genuine	Mature genuine	Bombay II	Hardwar	Rishikesh	Bombay I	Lucknow I	Lucknow II	Calcutta I	Calcutta II
40% NaOH + Lead acetate	Light brown with orange tinge	Purple	Light brown with orange tinge	Purple	Purple	Brown	Reddish-brown	Olive green	Brown	Reddish-brown
Sudan III	No change	Orange	Orange	Orange	Orange	Light brown	Brown with orangish-red tinge	Blackish-green	Brown	Brown
10% NaOH + $CuSO_4$	No change	Dark purple	Red	Dark purple	Dark purple	Reddish-brown	Green	Green	Brown	Brown
Acetic acid	No change	Dark orange	Yellowish-orange	Dark orange	Dark orange	Orangish-brown	Black	Black	Black	Black
5% iodine solution	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black
5% $FeCl_3$ solution	Blakish-green	Blackish-green	Blackish-green	Blackish-green	Blackish-green	Dark brown	Bottle green	Bottle green	Bottle green	Light brown with greenish-tinge
Conc. HCl + phloroglucinol	Red	Dark red	Dark red	Dark red	Dark red	Yellowish-brown	Dark purple	Dark purple	Blackish-brown	Reddish-brown
conc. HNO_3	Brown	Dark brown	Dark brown	Dark brown	Dark brown	Chocolate brown	Black	Brown	Orangish-brown	Reddish-brown
Saturated picric acid	Dark yellow	Dark orange	Dark yellow	Dark orange	Dark orange	Brown	Chocolate brown with green tinge	Bottle green	Dark brown with orange tinge	Brown with orange tinge
50% KOH	Yellowish-orange	Reddish-violet	Reddish-orange	Reddish-violet	Reddish-orange	Dark brown with yellow tinge	Black	Black	Dark brown	Purplish-brown

Table 2. Fluorescence analysis of powdered drug genuine and market samples.

	Colour under UV light									
Powder +	Young genuine	Mature genuine	Bombay II	Hardwar	Rishikesh	Bombay I	Lucknow I	Lucknow II	Calcutta I	Calcutta II
Powder as such	Light brown	Yellow	Brown	Brown	Green	Green	Reddish-brown	Black	Dirty brown	Brown
Nitro-cellulose in amylacetate	Dirty brown	Light orange	Pale yellow	Brown	Brown	Parrot green	Parrot green	Green	Green	Brown
1N NaOH aqueous soln	Dark brown	Reddish-orange	Orange	Reddish-orange	Reddish-orange	Parrot green	Purplish-red	Dull brown with green tinge	Dark brown	Dark brown
1N NaOH aqueous dried and mounted in nitrocellulose in amylacetate	Brown	Dark orange	Orange with green tinge	Orange with green tinge	Dark orange	Dull green	Purplish-red	Dark brown	Dark bown	Purple with green tinge
1N NaOH in methanol	Brown	Orange	Yellowish-orange	Orange	Orange	Brown with green tinge	Reddish-brown	Blackish-brown	Blackish-brown	Purple with green tinge
1N NaOH in methanol dried and mounted in nitrocellulose in amylacetate	Brown	Dark green	Dark green	Dark green	Dark green	Green	Reddish-brown	Blackish-green	Blackish-brown	Purple with green tinge
1N HCl	Dirty green	Orange with green tinge	Pale yellow	Orange with green tinge	Orange with green tinge	Dirty green	Brown	Blackish-brown	Blackish-brown	Dirty green
1N HCl dried and mounted in nitrocellulose in amylacetate	Dirty green	Orange with green tinge	Pale yellow	Orange with green tinge	Orange with green tinge	Green	Reddish-brown	Dark brown	Green	Dirty green
50% HNO_3	Black	Reddish-brown	Light brown	Reddish-brown	Reddish-brown	Green	Reddish-brown	Dark brown	Green	Dirty green
50% H_2SO_4	Reddish-brown	Light orange with green tinge	Pale yellow	Light orange with green tinge	Light orange with green tinge	Violet	Brown	Black	Black	Purple

Table 3. Preliminary phytochemical screening of 'sappan' wood.

		Samples									
Extractive	Constituents	YG	G	B II	R	H	BI	LI	LII	CI	CII
Hexane	Steroids	+	+	+	+	+	+	−	−	+	+
	Triterpenoids	+	+	+	+	+	−	+	+	−	+
	Resins	−	−	−	−	−	−	−	+	−	−
Benzene	Steroids	+	+	+	+	+	+	−	−	+	+
	Triterpenoids	+	+	+	+	+	−	+	+	+	+
	Resins	−	−	−	−	−	−	−	+	−	+
Chloroform	Resins	−	−	−	−	−	+	−	+	−	−
	Alkaloids	+	+	−	−	−	−	−	−	−	+
	Reducing sugars	+	+	+	+	+	+	−	−	+	+
Alcohol	Resins	−	−	−	−	−	+	−	+	−	−
	Tannins	+	+	+	+	+	+	−	+	+	+
	Flavonoids	−	−	−	−	−	+	+	−	+	−
	Reducing sugars	+	+	+	+	+	+	+	+	+	+
Water	Tannis	+	+	+	+	+	+	+	+	+	+
	Reducing sugars	−	+	+	+	+	+	+	+	+	−

The successive extractives were further screened (Peach and Tracy 1955) for steroids and triterpenoids (LB test), flavonoids (Shinoda's test), alkaloids (Mayer's reagent), tannins (ferric chloride test) and sugars (Fehling solution test) and the results are presented in table 3.

Comparative thin layer chromatography of successive extractives of the above samples was also carried out. However, no meaningful conclusions could be drawn on this basis.

8. Discussion and conclusions

A detailed study of the genuine drug and the market samples of Sappan procured from various parts of the country—Bombay, Calcutta, Hardwar, Lucknow and Rishikesh—was carried out.

The genuine Sappan, *Caesalpinia sappan* Linn., is characterised by: (i) diffuse porous wood (ii) growth rings demarcated by fine interrupted lines of parenchyma (iii) vessels very small, mostly open or sometimes filled with yellow deposits (iv) parenchyma vesicentric and (v) rays fine. It was observed that of all the market samples studied only those from Bombay (II), Rishikesh and Hardwar resembled the genuine drug in all the above characters and as such are identified as *C. sappan*.

An attempt was also made to identify the remaining samples. A search of old Ayurvedic literature revealed that Sappan has sometimes also been equated with Raktasar, a drug identified as *Pterocarpus santalinus* (Dwivedi 1949; Gor 1935). Keeping this in view it was observed that (i) diffuse porous wood with a tendency to

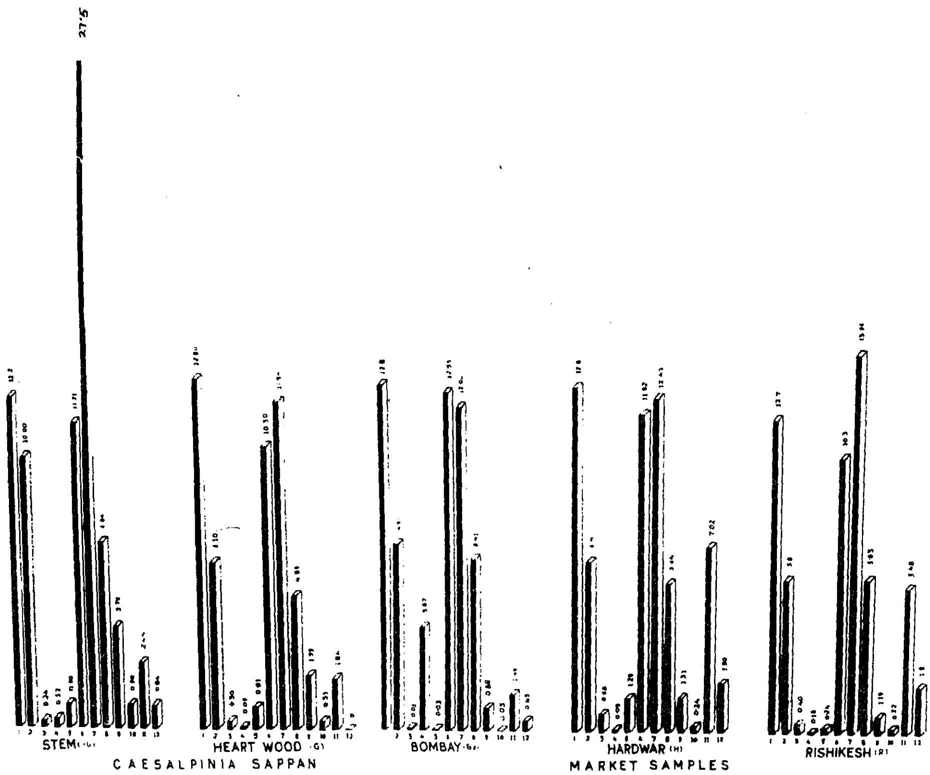
semi-ring porous condition (ii) parenchyma tissue predominantly in wavy bands (iii) vessels large and filled with reddish gummy deposits noticed in three of the market samples—Lucknow I, Lucknow II and Bombay I—are also found in the wood of *Pterocarpus* spp. It was, therefore, concluded that possibly all three belong to the genus *Pterocarpus*.

Further, Lucknow II could be differentiated from Bombay I and Lucknow I in having the following characters: (i) orange-red or purplish black coloured wood (ii) presence of ripple marks (iii) interlocked grains and (iv) a heavily impregnated fibrous portion with reddish brown gum, containing a red dye soluble in alcohol and ether but insoluble in water. Among the different *Pterocarpus* spp. similar characters have been reported in the wood of *P. santalinus* Linn. Lucknow II sample was, therefore, conclusively identified as *P. santalinus* Linn.

Again, Lucknow I sample differs, from Bombay I in having (i) deep reddish brown colour (ii) more pronounced semi-ring porous tendency and (iii) a faint yellowish blue fluorescence in cold water extractive. A systematic comparison of various *Pterocarpus* species showed similarities between *Pterocarpus dalbergioides* Roxb. and Lucknow I sample in all the above characters and was, therefore, identified as such.

The remaining sample—Bombay I—was identified as *P. marsupium* Roxb. in view of its resemblances with that species in having: (i) medium coarse-textured wood and (ii) distinct yellowish blue fluorescence in cold water extractive.

Samples procured from Calcutta market are totally different. Of these Calcutta I



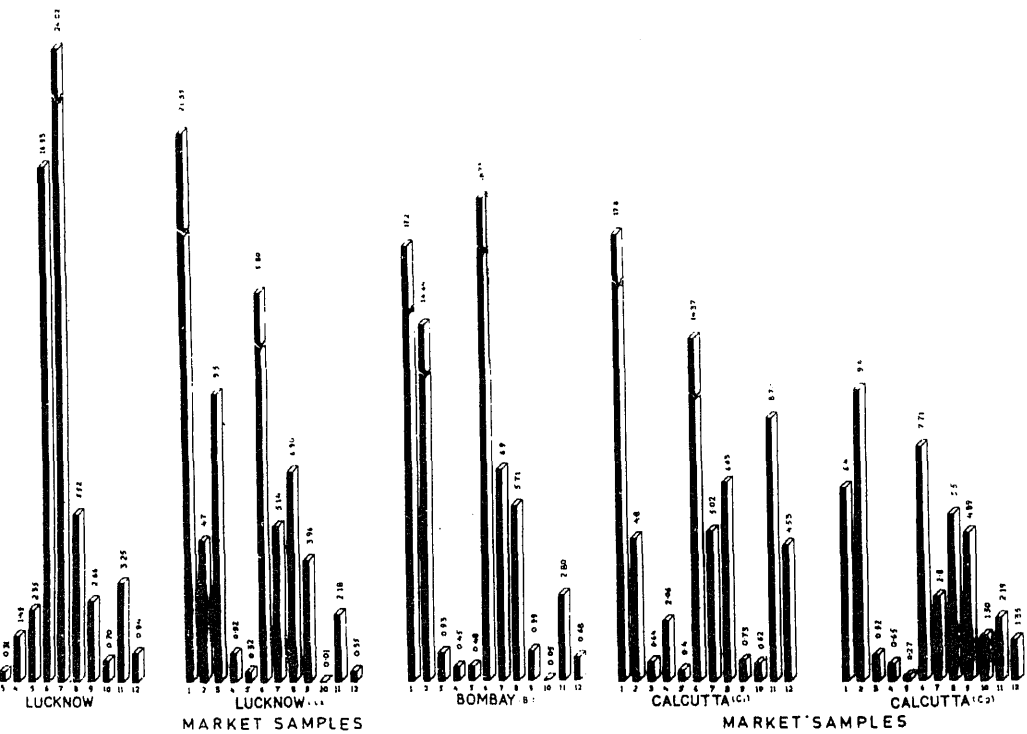


Figure 65. Percentages of physico-chemical constants. **1.** Alcohol soluble extractive (IP method). **2.** Water soluble extractive (IP method). **3.** Hexane soluble extractive. **4.** Benzene soluble extractive. **5.** Chloroform soluble extractive. **6.** Alcohol soluble extractive. **7.** Water soluble extractive. **8.** Loss on drying. **9.** Total ash. **10.** Acid in-soluble ash. **11.** Gallotannins. **12.** Total crude tannins.

sample is characterised by the presence of (i) schizogenous, horizontal and solitary gum canals arranged in fusiform rows (ii) heterogenous medullary rays (iii) solitary vessels and (iv) scarlet red colour. Calcutta II sample, however, shows (i) semi-ring porous to ring porous wood (ii) distinct growth rings (iii) vessels large, partly filled with tyloses or occasionally with yellowish white deposits and (iv) broad rays. On matching these characters with descriptions available from the studies of Rao and Juneja (1971), it was found that characters of Calcutta II sample resemble with *Toona ciliata* M. J. Roem. (Meliaceae) and that of Calcutta I with *Gluta travancorea* Bedd. These were accordingly matched respectively with genuine *Gluta travancorea* and *Toona ciliata* wood samples at FRI Dehra Dun for confirmation. Their identity was thus confirmed as *Gluta travancorea* Bedd. and *Toona ciliata* M. J. Roem. respectively.

A composite profile of the samples on the basis of their physico-chemical constants is presented in the form of histograms (figure 65). A summary of microscopical characters of the various samples is presented in table 4.

Table 4. Summary of microscopical characters of 'sappan' wood genuine and market samples.

Parameters	Young genuine	Mature genuine	Bombay II	Hardwar	Rishikesh	Bombay I	Lucknow I	Lucknow II	Calcutta I	Calcutta II
Nature of wood										
Storied	–	–	–	–	–	+	+	+	–	–
Non-storied	+	+	+	+	+	–	–	–	+	+
Pores										
Diffuse porous	+	+	+	+	+	–	–	–	+	+
Semi-ring porous	–	–	–	–	–	+	+	+	–	–
No. of vessels/mm^2	–	5–19	5–26	5–19	5–26	1–13	19–54	4–14	2–9	4–10
Tyloses	–	–	–	–	–	–	–	–	+	+
Type of rays										
Homogenous	–	–	–	–	–	+	+	+	–	–
Heterogenous	+	+	+	+	+	–	–	–	+	+
Height	2–21	3–38	3–38	4–18	4–18	4–10	4–20	4–10	10–25	2–25
Width	1–4	1–4	1–4	1–4	1–4	1–2	1–4	1–2	1–4	2–4
Gum canals	–	–	–	–	–	–	–	–	+	±
Fibres										
Septate	+	+	+	+	+	–	–	–	–	–
Non-septate	–	–	–	–	–	+	+	+	+	+
Gelatinous	+	+	+	+	+	–	+	–	+	–
Substitute	–	–	–	–	–	+	±	–	–	–
Stone cells	+	+	+	+	+	–	–	–	–	–

+ Present; ± Occasionally present; – Absent.

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The authors are grateful to Dr T N Khoshoo, ex-Director, NBRI for his keen interest during this work. They are thankful to Dr (Mrs) Usha Shome for chromatography of the above samples and several valuable suggestions. They are also thankful to Dr Ramesh Dayal of Forest Research Institute, Dehra Dun for help in confirming the identity of the market samples. Their thanks are also extended to Mr A P Dhiman for preparing the plates.

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Abbreviations (figures 1–64): CC, cell contents; CCM, corkcambium; COL, collenchyma; COR, cortex; CR, crystal; EP, epidermis; FIB, fibre; G, gum canal; GFIB, gelatinous fibre; MPR, metatracheal parenchyma; MR, meduallary rays; P, pittings; PAR, parenchyma; PH, phloem; PHF, phloem fibre; PHR, phloem rays; PI, pith; PPR, paratracheal parenchyma; SCL, sclerenchyma; STC, stone cell; TY, tyloses; VE, vessels; XY, xylem; XYF, xylem fibre; XYP, xylem parenchyma; XYR, xylem rays.

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Pharmacognostic studies on *Artemisia scoparia* Waldst. and Kit.*

USHA SHOME, PAMMIE JOSHI and H P SHARMA

Pharmacognosy Section, National Botanical Research Institute, Lucknow 226001, India

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Abstract. Pharmacognosy of the floral and vegetative parts of *Artemisia scoparia* Waldst. and Kit. ('Dona'), a bitter aromatic herb used in the indigenous systems of medicine, was carried out with a view to lay down standards for the genuine drug. The parameters studied include anatomical and microscopic details of the different parts; certain physico-chemical constants such as ash values, percentage of tannins, and successive extractives; as well as qualitative detection of the main chemical constituents in the various extractives. Thin layer chromatography and fluorescence tests of drug powder were also carried out. Presence of casparian strips in the endodermis, resin canals, four- to eight-celled characteristic glandular hairs on the corolla and absence of pappus are some of the diagnostic features of the drug.

Keywords. *Artemisia scoparia*; pharmacognosy; Asteraceae; casparian strips

1. Introduction

Artemisia scoparia Waldst. and Kit., known as 'Chauri Saroj' and 'Danti' in Bombay, 'Dona' and 'Jhan' in Punjab, is a bitter, aromatic herb. An infusion of the whole plant is used as a purgative (Steward see Kirtikar and Basu 1933) in the indigenous systems and as a cure for earache (Hughes Buller see Kirtikar and Basu 1933). The smoke of the plant is also considered good for burns (Kirtikar and Basu 1939).

The medicinal importance of *A. scoparia* has been brought out in several papers. Maksudov and Pogorelko (1963), Darenkov (1965), and Maksudov and Koisman (1966) discovered analgesic, diuretic, bactericidal, spasmolytic and sedative action of 'Artemisol', a Soviet compound preparation containing essential oils from *A. scoparia*.

Pavelkovskaya *et al* (1967) studied in detail the flavonoids of three sub-genera of *Artemisia*. Alyukina and Ryachovskaya (1980) concluded that *A. dracunculus*, *A. scoparia*, and *A. albicerata* are the most promising sources of some of the widely used flavonoids in medicine and some branches of industry.

In addition, Jamwal *et al* (1972) have shown pharmacological action of 6, 7-dimethoxy coumarin, (scoparine) isolated from *A. scoparia*, on experimental animals.

Despite its medicinal importance pharmacognosy of *A. scoparia* seems to have escaped the attention of pharmacognoscists. Thus, except for a paper by Nautial and Purohit (1980) who studied high altitude acclimatization, stomatal frequency and anatomical changes in leaves of *A. scoparia*, *A. vulgaris*, *A. parviflora* and *A. vestita*, no other information on its pharmacognosy is available and hence the present study.

2. Material and methods

The present studies were carried out on preserved material collected from Ladakh

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during September-October. Shade-dried material was used for fluorescence and chemical studies. Hand sections were cut and stained in safranin as well as in phloroglucinol solution followed by a few drops of concentrated hydrochloric acid. For leaf studies, however, microtome sections were used and stained with safranin and Fast Green combination. Ferric chloride, iodine, and Sudan III were used for testing tannin, starch and oil respectively. Schultze's fluid was used for macerations. For powder study the drug powder was cleared in chloral hydrate, stained in safranin and mounted in glycerine.

3. External characters

Artemisia scoparia is a 30–90 cm high, faintly odorous annual or biennial herb. The root is fusiform and branched and the stem simple at the base much branched above, grooved, purplish and hairless. Leaves are of two types (i) radicle ones long petioled, 2·5–7·6 cm long and decompound (ii) cauline leaves filiform. Floral heads are sessile or borne on short capillary peduncles in slender panicled racemes (figure 1).

4. Histological characters

4.1 *Stem*

A TS of the stem appears circular in outline and is ridged and furrowed alternately (figure 2). The epidermis is highly cuticularised and consists of rectangular cells measuring $7{\cdot}5$–$12{\cdot}5 \times 5$–$12{\cdot}5\ \mu m$. Some of these contain brownish, tanniniferrous contents. The cuticle is $2.5\ \mu m$ thick and has a smooth surface. The trichomes are uniseriate multicellular and *T*-shaped—similar to those reported in *Olearia dentata* Moench (William *et al* in Metcalfe and Chalk 1979). The cortex is chlorenchymatous and 3–4 layered (figures 3, 4B and 5). However, wedge-shaped collenchymatous mechanical tissue develops just below the ridges (figure 3). In the region of furrows, on the other hand, an arc of tissue develops into plain parenchyma below the chlorenchymatous layers (figure 4A).

The endodermis is very well-defined and consists of barrel-shaped cells (15–$16{\cdot}5 \times 7{\cdot}5$–$15\ \mu m$) with very prominent casparian strips on the lateral walls. The pericycle is composed of sclerenchymatous cells with very narrow lumen (figure 5) and is 5 to 10 layers in thickness. Opposite to the furrows pericycle is, however, 2–3-layered or is completely absent. The phloem is not very well-developed and occurs in patches in older stems (figures 2 and 3). The xylem is very well-defined and consists of vessels (figure 6A–C), tracheids (figure 6D–G), xylem fibres (figure 6H–K) and xylem parenchyma. The pith is large and composed of round, collenchymatous cells measuring 20–$62{\cdot}5\ \mu m$ (figures 3 and 6 L). Rhomboidal calcium oxalate crystals are present frequently in the pith cells (figures 3 and 6 O).

4.2 *Leaf*

4.2a *Petiole*: A TS of the petiole of a radicle leaf appears winged (figure 7). The epidermis is highly cuticularised and is composed of round to rectangular cells. The ground tissue is parenchymatous, except on either side of the median bundle where the

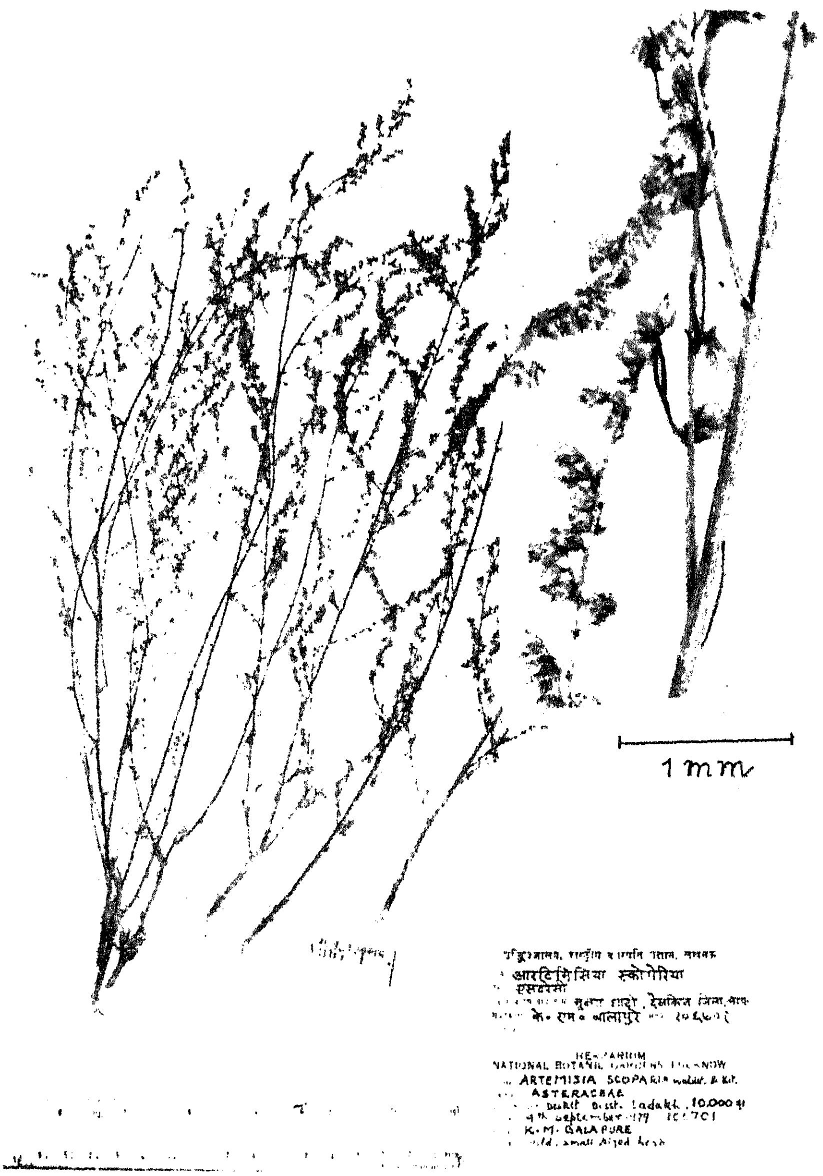
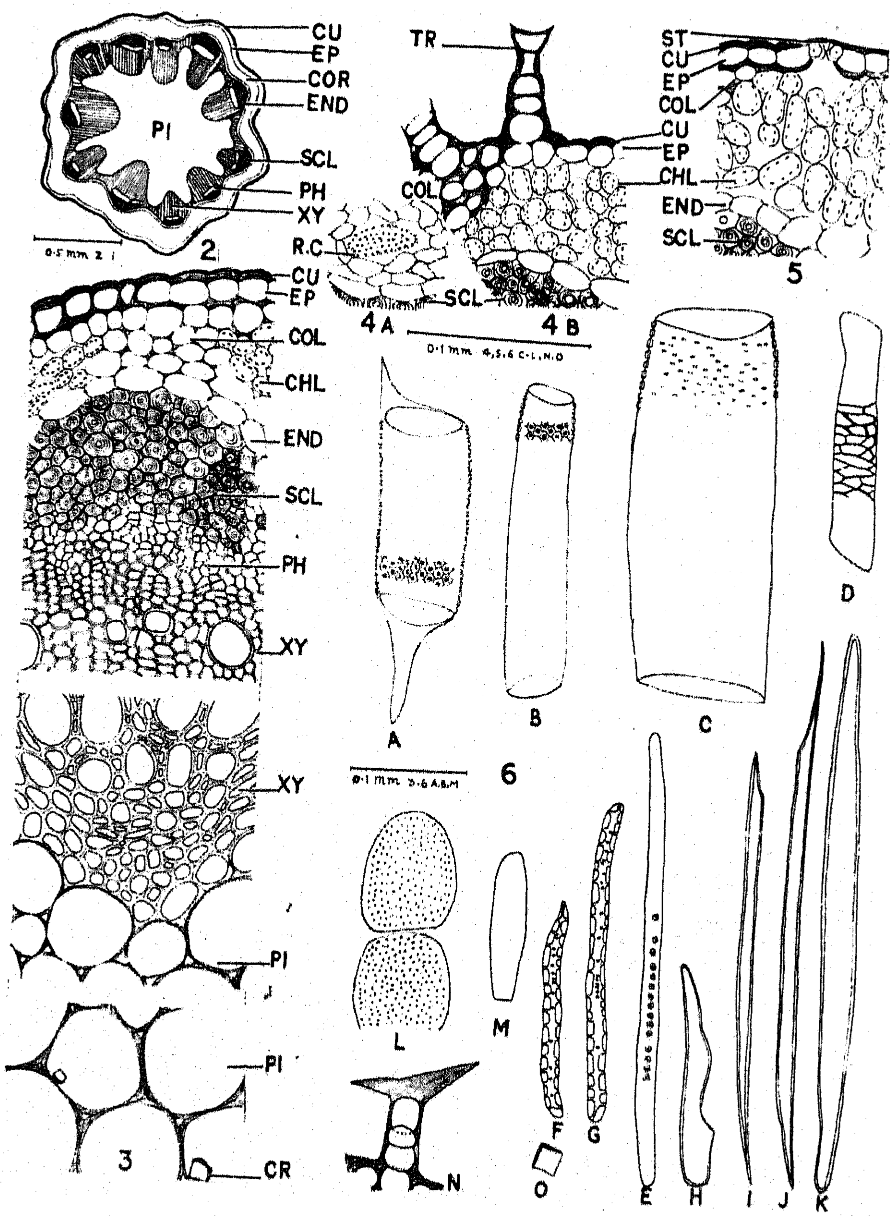


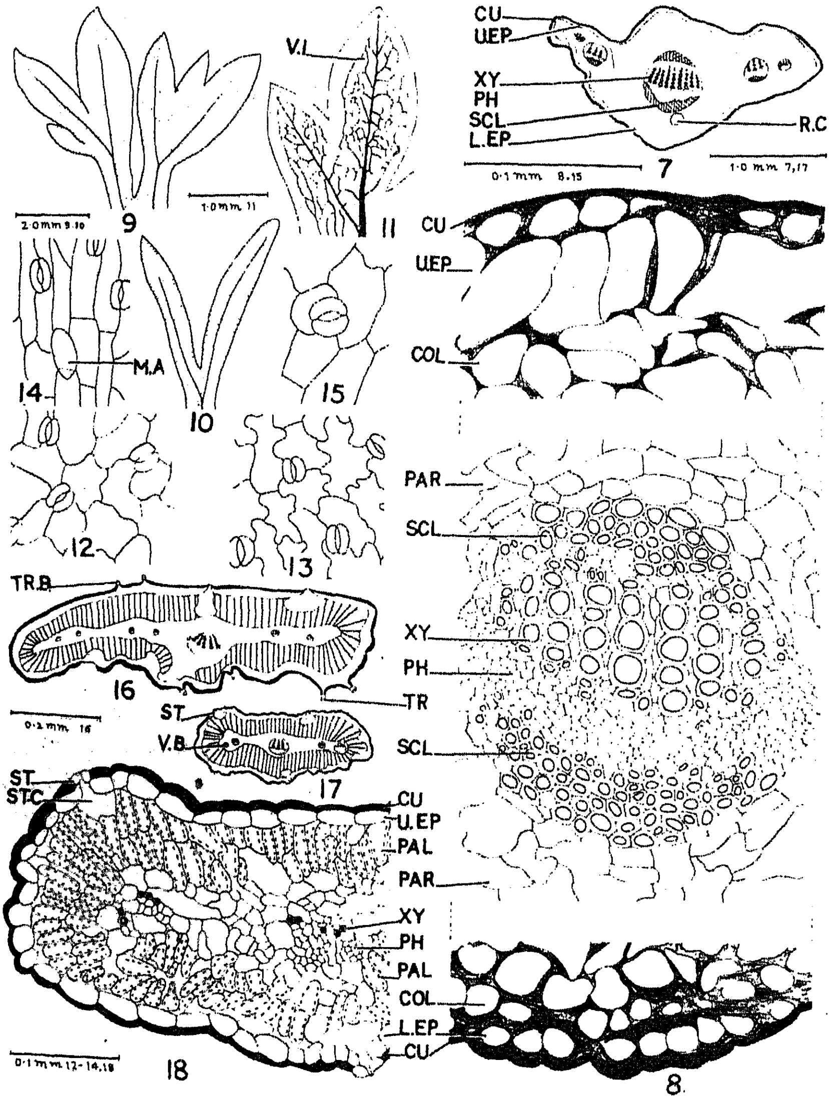
Figure 1. *Artemisia scoparia*, whole plant.

eripheral 2 to 3 layers are collenchymatous (figure 8). There are usually 3 to 5 collateral ascular bundles in the petiole, of which the median is the largest and 1 or 2 bundles ccurring in the wing are the smallest. The sclerenchyma is present as bundle caps on he dorsal and ventral sides of the median bundle and gradually decreases with the size f the veins, being entirely absent in the lateral bundles (figure 7). The xylem and hloem are both well-developed (figure 8). However, resin canals occur associated sually with the vascular bundles (figure 7).



Figures 2–6. **2.** T S of stem (diagrammatic). **3.** A portion from figure 2 showing details. **4A.** A portion of parenchymatous cortex showing a resin canal. **4B.** A portion of cortex showing trichomes. **5.** A portion of cortex showing stoma. **6A–O.** Isolated elements (for details refer to the text).

4.2b *Lamina:* Radicle and cauline leaves are differently dissected (figures 9 and 10 respectively) but both have reticulate venation (figure 11). No further differences were observed in their surface structure. The following description, therefore, holds good for both types of leaves.



Figures 7–18. **7.** T S petiole (diagrammatic). **8.** A portion of the same from its middle showing details. **9, 10.** Radicle and cauline leaves respectively. **11.** Lobules showing venation pattern. **12, 13.** Cells from the upper and lower epidermis respectively showing stomata. **14.** Cells over the vein showing stomata and multicellular glandular hair. **15.** Anisocytic stoma. **16, 17.** T S of lobule from the positions marked a-a, b-b in figure 9. **18.** A portion from the margin of figure 17 showing details.

In a surface view cells of the upper epidermis are large and undulate in outline (figure 12). Those of the lower epidermis have, however, more wavy outlines (figure 13). Epidermal cells in the region below the lobules are narrow and elongated (figure 14). Multicellular glandular hairs are also present sparsely distributed on the leaf (figure 14). Stomata are usually anomocytic (figures 12 and 13) but anisocytic ones are also not uncommon (figure 15).

The leaves show an isobilateral structure. In transverse sections passing through the lobule of a radicle leaf (positions marked a–a and b–b in figure 9) the epidermis is highly cuticularised and the mesophyll consists of (i) palisade parenchyma present on either side and (ii) parenchyma having air cavities in between. The substomatal cavities are very large, irregular and usually extend up to the base of the palisade. A varying number of vascular bundles are present embedded in the parenchyma. Of these the median one, which is similar in details to the corresponding bundle of the petiole except in the absence of sclerenchymatous fibres, is the largest and those in the wings are much smaller (figures 16 and 17). The epidermis at the margin is formed of large highly cuticularised cells which are broader than long. The stomata are slightly raised and the palisade is 1 to 2 layers thick (figure 18).

5. Inflorescence

The capitula are minute and are arranged on racemosely branched axes (figure 19). The outer part of the capitulum consists of bracts arranged as usual. The bisexual flowers are placed in the centre and female flowers occur towards the periphery.

5.1 *Bracts*

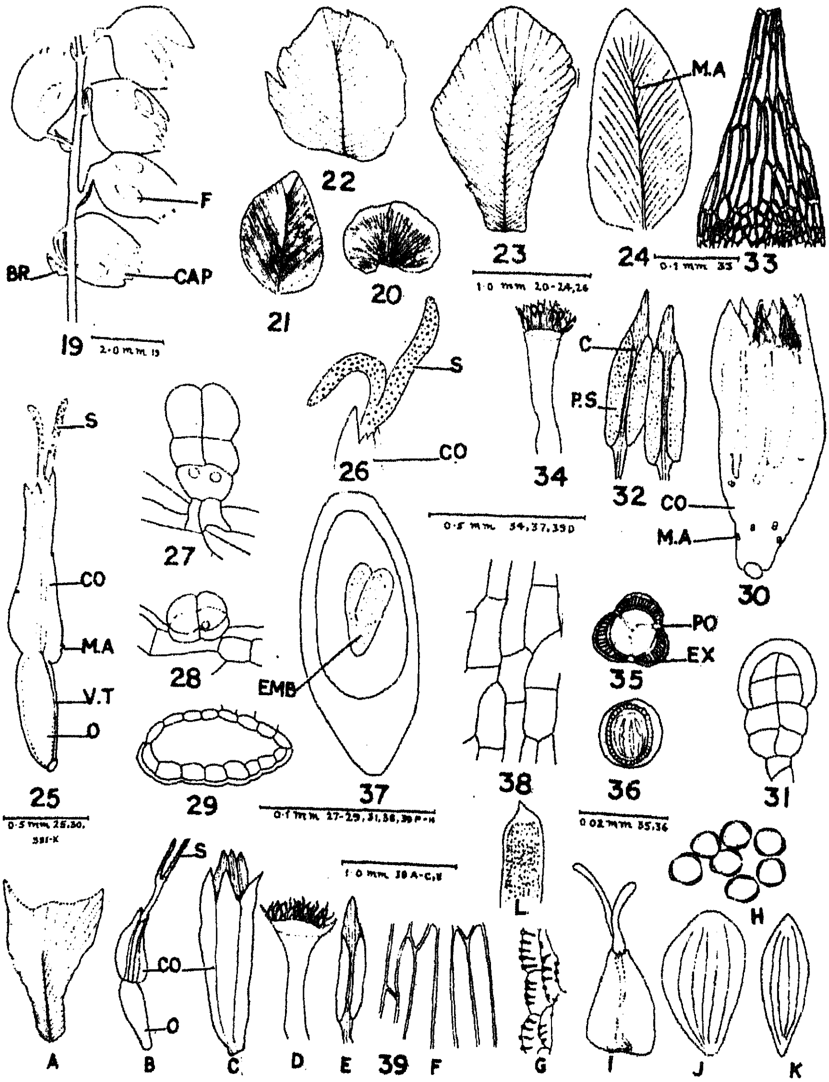
The inflorescence is surrounded by unequal involucral bracts, the outer ones being smaller (measuring 775–1425 μm) than the inner ones (1864·8–2331 μm). The bracts are glabrous and ovate to oblong, have broad scarious margins and are yellowish-green in colour. These consist of long elongated cells placed end-to-end, with a few stomata distributed irregularly. Multicellular glandular hairs are distributed sparsely on these structures (figures 20–24).

5.2 *Female flowers*

The flowers are fertile and have very minute and actinomorphic corolla (figure 25). The ovary is inferior and ovate. A long bifurcated stigma covered all over by receptive papillae (figures 25 and 26) is present. Multicellular glandular hairs, similar to those present on the leaf, are present on the corolla as well (figures 27 and 28). The basal cells of the ovary are arranged in the form of a ring (figure 29).

5.3 *Bisexual flowers*

These are arranged at the centre of the head. The corolla is much larger than that of female flowers, actinomorphic, 5-partite and covered all over by multicellular glandular hairs which are much more frequent here than on female flowers (figures 30 and 31). The anthers are longer than the corolla, syngenesious and the connectives are drawn out beyond the anther lobes (figures 30 and 32). The tips of the connectives are composed of vertically-elongated thick-walled cells (figure 33). Pollen sacs are two (figure 32). The gynoecium in bisexual flowers is very short and abortive. The style has a blunt apex and small papillate structures are present at the apex (figure 34).



Figures 19–39. 19. Capitula arranged in a racemose fashion on a floral branch. **20–24.** Involucral bracts of different shapes and sizes. **25.** A female flower showing inferior ovary and bifurcated stigma. **26.** Stigma enlarged. **27, 28.** Muticellular glandular hairs. **29.** Basal cells of the ovary. **30.** Bisexual flower. **31.** Multicellular glandular hair from the bisexual flower. **32.** Anther lobes enlarged. **33.** Apical cells of the connective which are drawn out beyond anther lobes. **34.** Sterile stigma. **35.** A pollen grain showing wall stratification. **36.** A palynogram of a pollen. **37.** Fruit showing an embryo. **38.** Epidermal cells from the surface of the fruit. **39.** A-L. Different tissues from the drug powder (for details refer to the text.).

Abbreviations: BR, bract; CAP, capitulum; CHL, chlorenchyma; CO, corolla; COL, collenchyma; COR, cortex; CR, crystal; EMB, embryo; END, endodermis; EP, epidermis; EX, exine; F, fruit, LEP, lower epidermis; MA, multicellular glandular hair; O, ovary; PAR, parenchyma; PH, phloem; PI, pith; PO, pore; PS, pollen sac; RC, resin canal; S, stigma; SCL, sclerenchyma; ST, stoma; STC, stomatal cavity; TR, trichome; UEP, upper epidermis; VT, vascular trace; XY, xylem.

5.4 *Pollen grains*

Pollen grains are usually oblate, spheroidal, prolate, 3-colpate, and measure 16 × 18 μm. Sexine consists of two parts: an inner endosexine and an outer ectosexine much thicker (1–2 μm) than the endosexine. Ornamentation is psilate (figures 35 and 36).

6. Fruit

The fruit is a very minute and ellipsoid achene, measuring 759–1000 μm (figure 37). The epidermal cells are longer than broad (figure 38).

7. Powder study

The powder is slightly greenish yellow with a faint odour and bitter taste. The powder was cleared in chloral hydrate and mounted in glycerine. On microscopic examination the following structures were observed.

(i) Entire and broken pieces of bracts (figure 39A). (ii) Female and bisexual flowers (figure 39B and C). (iii) Blunt stigma and style from female flower (figure 39D) (iv) Anther-lobe (figure 39E). (v) Cells from connective (figure 39F). (vi) Epidermal cells from the anther lobe (figure 39G). (vii) Pollen grains (figure 39H). (viii) Corolla and style from the female flower (figure 39I). (ix) Fruits (figure 39J and K). (x) A broken apical portion of a leaf (figure 39L).

On treatment with different reagents the powder gave positive tests for tannins, oils, sugars, strach etc (table 1).

The fluorescence of the powder was analysed according to the methods given by Chase and Pratt (1949) and Kokoski *et al* (1958) and the observations are recorded in table 2.

Table 1. Behaviour of powder on treatment with different chemical reagents in *A. scoparia*.

	Observation	
Powder treated with	Inflorescence	Vegetative shoot
Powder as such	Greenish brown	Pale brown
Picric acid (saturated)	No change	No change
Lactic acid	No change	No change
Conc. nitric acid	Yellowish brown	Orange brown
Conc. HCl	Olive green with brown tinge	Blackish brown
Conc. H_2SO_4	Blackish brown	Blackish brown
Acetic acid	Dirty brown	No change
Ferric chloride	Light green	Light green
Iodine	Brown	Reddish brown
Sudan III	No change	No change
10% NaOH followed by a drop of copper sulphate solution	Bottle green	Dull green

Table 2. Fluorescence analysis of powdered drug in *A. scoparia*.

Treatment powder plus	Colour under day light		Colour under UV light	
	Inflorescence	Vegetative shoot	Inflorescence	Vegetative shoot
Powder as such	Greenish brown	Pale brown	Dark brown	Pale brown
Nitrocellulose in amyl acetate	No change	No change	Brown with purple tinge	Dark brown
1N HCl	No change	No change	Blackish brown with green tinge	Dark brown with green tinge
1N HCl, dried and treated with nitrocellulose in amyl acetate	No change	No change	Brown with purple tinge	Greenish brown
1N NaOH in water	Brown	Yellowish brown	Brownish green	Olive green
1N NaOH in water dried and treated with nitrocellulose in amylacetate	Yellowish brown	Yellowish brown	Olive green	Olive green
1N NaOH in methanol	Yellowish brown	Yellowish brown	Greenish brown	Greenish brown
1N NaOH in methanol dried and treated with nitrocellulose in amylacetate	Yellowish brown	Yellowish brown	Greenish brown	Greenish brown
50% HNO_3	Brown	Orange brown	Greenish brown with black tinge	Greenish brown with black tinge
50% H_2SO_4	Greenish brown	Greenish brown	Greenish brown	Greenish brown

8. Phytochemical studies

As an aid to further characterisation of the drug, the ash and tannin percentages were determined on air dry weight basis according to recommended procedures (Anon 1965, 1966) and alcohol and water extractive by IP methods respectively. The results are presented in table 3.

A known quantity of powdered *Artemisia* flowers was extracted in a soxhlet apparatus with hexane, benzene, chloroform, alcohol and water successively and the percentage of each extractive was calculated from the respective extractives by evaporating the solvents. These were further tested (Peach and Tracy 1955) for steroids and triterpenoids (L B test); flavonoids (Shinodas test); alkaloids (Mayer's reagent) and tannins (Ferric chloride test). The results obtained are summarised in table 4.

Unidirectional chromatography of the various extractives presented the following picture.

8.1 *Hexane extractive*

Using pure benzene as a solvent the hexane extractive in inflorescence gave 5 spots at the base, two in the middle and a semilunar spot near the solvent front whereas in vegetative shoot, it gave 9 clear spots at the base, one in the middle and one near the solvent front (figure 40).

8.2 *Benzene extractive*

Benzene extractive in the inflorescence and vegetative shoot gave almost similar results while using chloroform as the solvent except in inflorescence a bright, yellow spot separates out near the base and 3 very close spots—one light brown and two light purple—a little higher up (figure 41).

8.3 *Chloroform extractive*

Chloroform extractive gave the best results using 100 ml chloroform and 0·2 ml acetic

Table 3. Determination of ash and tannin on air dry weight basis

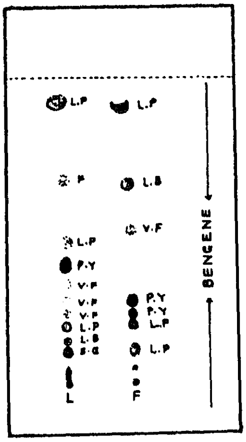
	Inflorescence (%)	Vegetative shoot (%)
Ash		
Total ash	7·611	2·5269
Acid insoluble ash	3·040	1·2120
Tannins		
Crude tannins	3·381	0·337
(calculated as Gallotannins)	0·622	0
Alcohol soluble extractive	9·1	12·2
Water soluble extractive	32·6	32·7

Table 4. *Artemisia scoparia* Waldst. & Kit. Preliminary phytochemical tests.

Parameters	Hexane		Benzene		Chloroform		Alcohol		Aqueous	
	I	VS	I	VS	I	VS	I	VS	I	VS
Total weight (%)	4·280	1·23	2·615	1·35	1·820	0·61	14·5	14·27	11·77	9·55
Physical appearance and consistency	Light yellowish green, waxy, pleasant smell	Dark olive green, waxy, no smell	Dark yellowish brown, waxy, pleasant smell	Olive green, dry, pleasant smell	Yellowish brown, dry, pleasant smell	Yellowish olive green, dry, no smell	Reddish brown, dry, sugary smell	Reddish brown, tachy, pleasant smell	Brown, dry, no smell	Blackish brown, dry, no smell
Steroids	+	+	+	+	+	+	−	−	−	−
Triterpenoids	−	+	−	+	−	+	−	−	−	−
Alkaloids	−	−	−	−	−	+	+	+	+	±
Flavonoids	−	−	−	−	−	−	+	+	+	−
Tannins	−	−	−	−	−	−	+	+	+	+
Reducing sugars	−	−	−	−	+	−	+	+	+	+
Polysaccharides/ glycosides	−	−	−	−	−	−	−	−	−	−
Saponins	−	−	−	−	−	−	−	+	+	+
Resins	−	−	−	−	−	−	−	−	−	−

Total percentage of extractive inflorescence = 34·985 %; Vegetative shoot = 26·01 %

I = Inflorescence; VS = vegetative shoot



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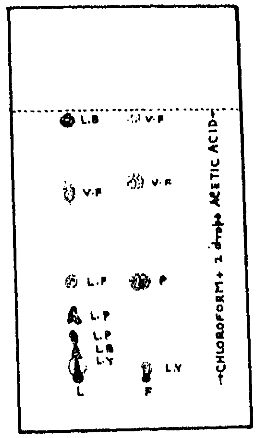

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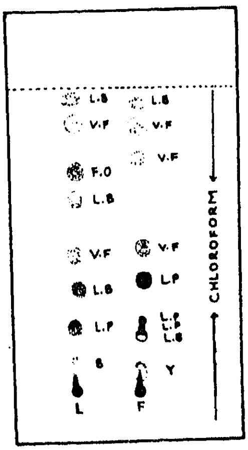

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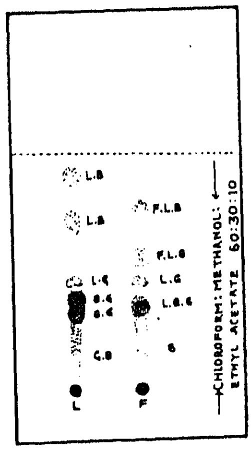

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Figures 40–43. Chromatograms of hexane, benzene, chloroform and alcohol extractives respectively.
Abbreviations: B, brown; BG, bottle green; F, faint; FLB, faint light brown; FO, faint orange; GB, greenish brown; LB, light brown; LBG, light brownish green; LG, light green; LP, light purple; LY, light yellow; PY, purple yellow; SG, steel grey; VF, very faint; L, vegetative shoots; F, inflorescence.

acid as a solvent. In vegetative shoot 5 spots separated out near the solvent front; whereas in inflorescence only 2 spots—one near the point of origin and the other a little higher up—were present. The other spots were similar in both the cases (figure 42).

8.4 *Alcohol extractive*

Several solvents were tried for alcohol extractive. However, the best results were obtained with chloroform : methanol : ethyl acetate (60 : 30 : 10) + 0·2 ml of acetic acid.

Two characteristic dark bottle green spots develop in vegetative shoot a little higher from the point of origin. Only one such spot, slightly lighter in colour develops at the same height as in inflorescence. An additional light brown spot near the solvent front is present in the inflorescence.

9. Discussion

Pharmacognosy, which developed as a science, nearly two centuries ago, to check adulteration and quality of drugs, spices and other natural products imported by the European countries, mostly from the Tropical ones, has since changed in emphasis from alpha pharmacognosy to detection, isolation, characterisation and synthesis of naturally occurring compounds as emphasis changed, over the years, from natural products to synthetic drugs. In the Indian context, however, alpha pharmacognosy remains still valid as ever since the indigenous systems are almost exclusively dependent on herbal crude drugs which are highly prone to adulteration and substitution. It is, therefore, of utmost importance that standards are evolved which can help to distinguish the genuine drugs from their adulterants and substitutes. Botanical studies can play a very significant role in this direction as has been demonstrated by several workers including the present authors (Shome *et al* 1979; 1980a, b; 1981, 1982).

In this context herbaceous habit of *Artemisia scoparia* along with minute, filiform, decompound, cauline leaves; absence of pappus in the flower; presence of resin canals lined with well-demarcated glandular epithelium in the cortex of the stem and midrib region of the leaf, casparian thickenings in the endodermal cells, sclerenchymatous pericycle, again in the stem; isobilateral mesophyll in the leaves; characteristic glandular hairs with several-celled heads on petals and connectives drawn beyond anther lobes, as also TLC patterns of different extractives appear distinctive enough to be considered diagnostic in value. These characters can, therefore, be used to identify the drug from its possible adulterants.

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Taxonomy of *Bidens* section Psilocarpaea (Asteraceae-Heliantheae-Coreopsidinae) in India

K M M DAKSHINI and PRITHIPALSINGH
Department of Botany, University of Delhi, Delhi 110 007, India

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Abstract. Critical examination of numerous specimens showed that *Bidens* sect. Psilocarpaea is represented in India only by *B. pilosa* var. *β minor* (Bl.) Sherff and *B. bipinnata* Linn. The occurrence of *B. biternata* (Lour.) Merr. & Sherff as suggested earlier could not be established during the present taxonomic investigation on the genus *Bidens* in India.

Keywords. *Bidens*; taxonomy; *Psilocarpaea*.

1. Introduction

Delimitation of taxonomic categories is primarily based on the correct appreciation of discontinuities in character variation and/or in patterns of geographical distribution. In stable taxa these requisites are generally fulfilled and the taxonomic units are easily definable. On the other hand taxonomic groups in which phenotypic variability and limited gene exchange exist pose a serious difficulty to the taxonomists. The genus *Bidens* Linn. belongs to this category (Gillett 1975; Gillett and Lim 1970; Mensch and Gillett 1972; Grierson 1972; Sherff 1937; Weedon *et al* 1974; Wild 1967). Although only three species of *Bidens* section Psilocarpaea (characterised by long slender cypselae narrowed from the middle to the top) namely *B. pilosa* L., *B. bipinnata* L., and *B. biternata* (Lour.) Merr. & Sherff have been reported to occur in India, their taxonomic treatments have varied from time to time, and the understanding of their circumscription is still disputed. According to some authors (Chavan and Oza 1961, 1966; Maheshwari 1963; Oomachan and Billore 1969; Rao and Joseph 1965; Rau 1968; Santapau 1953; Saldanha and Nicolson 1976), *Bidens biternata* (Lour.) Merr. & Sherff is the most common representative of this group, the Linnean *B. pilosa* is either absent or very rare and *B. bipinnata* L. is infrequent in its distribution. However, Babu (1977) reported that *B. pilosa* L. and *B. bipinnata* L. are frequently distributed in the Northwest Himalaya, and remarked that *B. biternata* "may occur within this area as a weed". A similar observation on the distribution of *B. pilosa* L. in S. India was made by Matthew (1981).

A comparative analysis of the taxonomic treatments in these floristic accounts clearly demonstrates that the problems related to the identification of these taxa have been largely due to the use of leaf morphology as the main taxonomic character (Babu 1977; Chavan and Oza 1961). In a taxonomic investigation such characters do not generally help in identification of a large number of specimens and the discontinuities are not appreciated.

Further, the taxonomic distinctness of these three species has been influenced by the views on the synonyms assigned to each taxon. The following brief resumé of the

available literature illustrates the various views on the circumscription and delimitation of taxa within the section Psilocarpaea. Clarke (1876) listed *Bidens pilosa* L. and *B. decomposita* DC. in his 'Compositae Indicae'. Hooker (1882) segregated *B. pilosa* L. into three varieties, *B. pilosa* var. 1 *pilosa* proper, var. 2 *bipinnata*, and var. 3 *decomposita*. Additionally, in his notes Hooker (1882) listed *Bidens wallichi* DC. as a synonym of *B. pilosa* var. 2 *bipinnata*. Duthie (1903) recognised only *B. pilosa* L. and listed *B. bipinnata* (i.e. *B. pilosa* var. 2 *bipinnata* sensu Hooker) as its synonym. Cooke (1906) considered Hooker's var. 2 *bipinnata* as 'scarcely worth distinction' in the Bombay Presidency, and thus recognised only *B. pilosa* L. as the common Indian member of the genus *Bidens*. Collett (1921), on the other hand, recognised *B. pilosa* L. and *B. wallichi* DC. as distinct species, thus not following Hooker (1882) who considered the latter as a synonym of *B. pilosa* var. 2 *bipinnata*.

Sherff (1937) in his monograph on the genus *Bidens* pointed out the erroneous treatment by Hooker (1882) of *B. bipinnata* as a variety of *B. pilosa* and instead established *B. bipinnata* L. as a distinct species with the following names as its synonyms: *B. pilosa* var. 2 *bipinnata* (L.) Hook.; *B. pilosa* var. 3 *decomposita* (Wall. ex DC.) Hook.; and *B. decomposita* Wall. ex DC. Sherff (1937) also removed *B. wallichi* DC. from the synonymy of *B. pilosa* var. 2 *bipinnata* of Hooker, and considered it as conspecific to *Bidens biternata* (Lour.) Merr. & Sherff. Further, in his notes under *B. bipinnata* (Sherff 1937 p. 372) and *B. biternata* (Sherff 1937 p. 399) he commented that due to the superficially intermediate leaves, '*B. biternata* (= *B. wallichi* DC.), a species very common in British East India', was erroneously equated by Hooker with *B. bipinnata*. Santapau (1953) overlooked 'British East India' in the above statement of Sherff (1937) and remarked: 'This (i.e. *Bidens biternata*) is the plant known to the writers of the Indian Floras as *B. pilosa*. Sherff in his recent monograph on the genus *Bidens* has shown that the common Indian plant is not the Linnean plant. Subsequently all the floristic accounts published by Indian taxonomists dealt with *B. biternata* (Lour.) Merr. & Sherff as the common Indian *Bidens* with *B. pilosa* auct. non Linn. as its synonym (Chavan and Oza 1961, 1966; Maheshwari 1963; Oomachan and Billore 1969; Rao and Joseph 1965; Rau 1968; Saldanha and Nicolson 1976).

In recent years some taxonomists who regard the common Indian plant as *Bidens biternata* (Lour.) Merr. & Sherff also reported the occurrence of the Linnean *B. pilosa* as a rare plant from several parts of the country (Chavan and Oza 1961, 1966; Oomachan and Billore 1969; Panigrahi *et al* 1964; Saldanha and Nicolson 1976; Santapau 1961). *Bidens bipinnata* L. has also been reported as a new record from different parts of the country (Babu 1977; Chavan and Oza 1966; Dakshini and Prithipalsingh 1971; Raizada 1976; Saldanha and Nicolson 1976). Thus the taxonomy and nomenclature of *B. pilosa*, *B. bipinnata*, and *B. biternata* in our country are in a confusing state. There is an important need to clear this confusion especially because these taxa form a weedy community. The present paper deals with the taxonomy and nomenclature of *Bidens* section Psilocarpaea in India.

2. Materials and methods

Following the suggestion that 'species and lower taxa should be formulated around natural populations' (Davis and Heywood 1967), plants were sampled from natural populations of *Bidens* taxa from different regions of India. These plants were examined

to establish the extent of variation in vegetative (particularly leaf morphology) and floral (capitulum morphology, organisation, phyllaries, cypselae, etc) characters. These studies were supplemented with a critical examination of herbarium specimens available in the major Indian herbaria, and also of photographs of authentic specimens located at Kew, Gray, Field Museum of Natural History, and British Museum (Natural History) herbaria. We have followed Sherff (1937) for typification of relevent taxa and location of type specimens.

3. Results and discussion

The specimens examined fall in two distinct groups. The characters studied in detail and the range of variation in these characters are summarised in table 1. Leaf morphology is highly variable (Figures 1–6). Even within these two groups different patterns could be identified based on the degree of dissection of the lamina and the shape of ultimate segments. It is amply clear from these data that such variable vegetative characters overlook discontinuities in more stable reproductive characters. It is primarily due to the reliance on leaf morphology that there has been an erroneous identification of the *Bidens* taxa by earlier workers. However, our observations show that it is easy to establish the range of distinct discontinuities in the characters of flowering and fruiting capitula and more particularly on the cypselar morphology, and thus highlight the use of these characters for the delimitation of *Bidens* species. A similar suggestion for delimiting the species of section Psilocarpaea was made by Sherff (1937) who emphasised the importance of the morphology of involucral bracts, number of total and ligulate florets per capitulum, cypselae morphology, and number of awns per cypsela. Unfortunately these suggestions did not influence a majority of Indian taxonomists. Similar to Sherff's (1937) observations, we have also found that the monomorphic cypselae is a very important feature for distinguishing *Bidens pilosa* L. from *B. bipinnata* L. and *B. biternata* (Lour.) Merr. & Sherff. The latter two species have dimorphic cypselae within a single capitulum. Of these, *B. biternata* has glabrous outer cypselae and hispid (but not tuberculate-hispid) inner ones, while in *B. bipinnata* the outer cypselae are tuberculate-hispid and the inner ones are sparsely hairy in the upper-half and glabrous in the lower-half. In the Indian *B. pilosa* L. all the cypselae in a capitulum are linear-curved, black, and tuberculate-hispid either apically or throughout their length. Similarly, in *B. bipinnata* L as represented in India, the dimorphic cypselae within a single capitulum are all linear, but differ in their size, colour and surface patterns. The outer cypselae which are tuberculate-hispid are generally brown in colour, but sometimes they may be hispid only and then blackish-brown. The inner ones are black and generally glabrous throughout their length or occasionally very sparsely hairy only in the apical region.

Recognising that variation in cypselar morphology is an important character for identification, and on the basis of a correlative assessment of characters listed in table 1 and taking the data presented by Sherff (table 2) into account, the two groups of plants commonly occurring in India conform to (i) *B. pilosa* var. *β minor* (Bl.) Sherff (group I plants in table 1) and (ii) *B. bipinnata* L. (group II plants in table 1). Contrary to earlier observations, none of the material now examined could be assigned to *B. biternata* (Lour.) Merr & Sherff as defined by Sherff (1937). In fact, the present observations support Sherff's comments regarding this taxon as 'a species very common in British

Table 1. Comparison of some vegetative and floral characters of *Bidens* taxa in India.

Character	Group I plants	Group II plants
Vegetative		
Leaf morphology	4 patterns: (a) All leaves entire (figure 1) (b) Lower leaves 1-segmented, upper leaves 3-segmented (c) All leaves 3-segmented (figure 2) (d) Leaves 3- and 5-segmented (figure 3)	3 patterns: (a) Leaves 5-, or 7-, or 9-segmented; segments ovate-lanceolate (figure 4) (b) Lower leaves 9- or less than 9-segmented, upper leaves more than 9-segmented; segments ovate-lanceolate (figure 5) (c) Leaves bipinnatisected; segments ovate-linear to linear (figure 6)
Petiole length (cm)	0·3–6	1–5
Lamina length (cm)	1·2–6·2	2·2–13
Lamina breadth (cm)	0·5–4·4	0·3–2·3
No. of serrations	5–20	0–10
Flowering capitulum		
Shape	Globose to subglobose	Cylindrical to elongated
Length (cm)	0·35–0·8	0·35–0·9
Breadth (cm)	0·25–0·6	0·2–0·9
Outer involucral bract		
(a) Length (cm)	0·30–0·60	0·2–0·8
(b) Shape	Spathulate to linear-oblong	Linear or subulate
(c) Vestiture	Pilose	Glabrous or pilose or glandular-pilose
(d) Margin	Ciliate	Ciliate
Inner involucral bract		
(a) Length (cm)	0·42–0·70	0·45–0·9
(b) Shape	Lanceolate	Lanceolate or oblong
(c) Tip	Obtuse	Obtuse or truncate
(d) Vestiture	Glabrous or pilose or glandular	Glabrous or pilose or glandular pilose
(e) Margin	Ciliate	Ciliate or glandular or ciliate-glandular
No. of ligulate florets/capitulum	3–7	2–5
Ligulate florets		
(a) Ovary length (mm)	0·8–1·5	0·5–2

(b) Ligule tube length (mm)	0·4–1·5	0·8–2·5
(c) Ligule limb length (mm)	3·7–9	2·2–5·2
(d) Ligule limb breadth (mm)	2–6	1·2–3
No. of disc florets/capitulum	25–58	5–24
Disc florets		
(a) Ovary length (mm)	1·0–2·5	1·2–3
(b) Corolla tube length (mm)	0·4–1·5	0·8–1·5
Fruiting capitulum		
Length (cm)	0·7–1·7	1·2–2·2
Breadth (cm)	0·5–2	0·3–1·2
Outer involucral bract length (cm)	0·3–0·75	0·3–0·8
Inner involucral bract length (cm)	0·42–0·70	0·45–0·9
Cypselae		
Morphology	Monomorphic, linear-curved, black, tuberculate-hispid	Dimorphic, linear, black or brownish-black or brown, glabrous or hispid-hairy or tuberculate-hispid
Length (cm)	0·6–1·7	1–2
Weight (mg)	0·6–3	1–7·1
Identification based on a correlative assessment of characters in comparison with data from Sherff (1937)	*Bidens pilosa* var. *β minor* (Bl.) Sherff	*Bidens bipinnata* Linn.

Figures 1–6. Photographs of herbarium specimens showing different leaf patterns observed in *Bidens pilosa* var. *β minor* (Bl.) Sherff **1.** *Aggarwal 10649* (DUH); **2.** *Panigrahi and Arora 8919* (BSA); **3.** *Aggarwal 6678* (DUH) and *Bidens bipinnata* Linn. **4.** *Irani NI 4604* (BLAT); **5.** *Prithipalsingh 10027* (DUH); **6.** *Aggarwal 10001* (DUH); (see text for explanations).

East India'. This region comprised only a very small portion of the North-east part of the present Republic of India but was mainly referable to other contiguous North-eastern regions such as Burma and the Malaysian peninsula. It may also be pointed out that this region formed part of the tract extending upto Canton-China, the type locality of *Coreopsis biternata* Lour. (the basionym of *Bidens biternata*). However, since this species has been reported to occur west of India also, further studies and collections are needed to document the occurrence and distribution of this species in India.

Table 2. Comparison of selected characters of *Bidens* in India with data from Sherff (1937) for identification of *B. pilosa* var. *β minor* and *B. bipinnata.*

Taxon / Character	Morphology of outer involucral bract	No. of florets/ capitulum	No. of ligulate/ florets	Cypselae
B. pilosa var. *β minor*				
(a) Data from Sherff (1937)	Linear to linear-spathulate	25–40	4–7	monomorphic; linear to sub-incurved, glabrous in lower half, tuberculate-hispid in upper half; 2 or 3 aristate
(b) Data from present investigation	Linear-oblong to spathulate	25–58	3–7*	monomorphic; linear to linear-curved; black; glabrous or not in lower half, tuberculate-hispid in upper half (outer ones sometimes tuberculate throughout); 2- or 3- (rarely 4-) aristate
B. bipinnata				
(a) Data from Sherff (1937)	Linear	+	+	dimorphic; linear, inner black; sparsely hairy in upper half, glabrous in lower; outer tuberculate-hispid (rarely 2-) 3- or 4-aristate
(b) Data from present investigation	Linear to subulate	5–24	2–5	dimorphic; linear; inner black, glabrous; outer blackish-brown and hispid or brown and tuberculate-hispid; 2- or 3- or 4-aristate
B. biternata				
Data from Sherff (1937)	+	20–30	3	dimorphic; black or brownish towards apex; linear; outer glabrous; inner hispid 4- (rarely 3- or 5-) aristate

* We have observed a minimum number of 3 ligulate florets per capitulum in the Indian material of *B. pilosa* var. *β minor* whereas Sherff reported 4 ligulate florets. Reduction in the number of ligulate (ray) florets has been reported as an evolutionary trend in this taxon (see Rajan 1975). + Data not available.

Thus *Bidens pilosa* var *β minor* (Bl.) Sherff and *B. bipinnata* L. are the commonly distributed taxa throughout India. The occurrence of *B. biternata* (Lour.) Merr & Sherff, as suggested earlier by many Indian taxonomists has not been substantiated. To facilitate easy identification of these species of *Bidens* in India, a key to the species and diagnostic characters along with the specimens examined are provided below.

4. Key to the species

A. Ligule white

B. Outer involucral bracts linear; cypselae dimorphic (outer glabrous, inner hispid); usually 4 (rarely 3 or 5) aristate . . . . *B. biternata*

BB. Outer involucral bracts spathulate; cypselae monomorphic, tuberculate-hispid; usually 2 or 3 aristate . . . . *B. pilosa* var. *β minor*

AA. Ligules yellow; cypselae dimorphic (outer tuberculate-hispid, inner sparsely hairy to glabrous) . . . . *B. bipinnata*

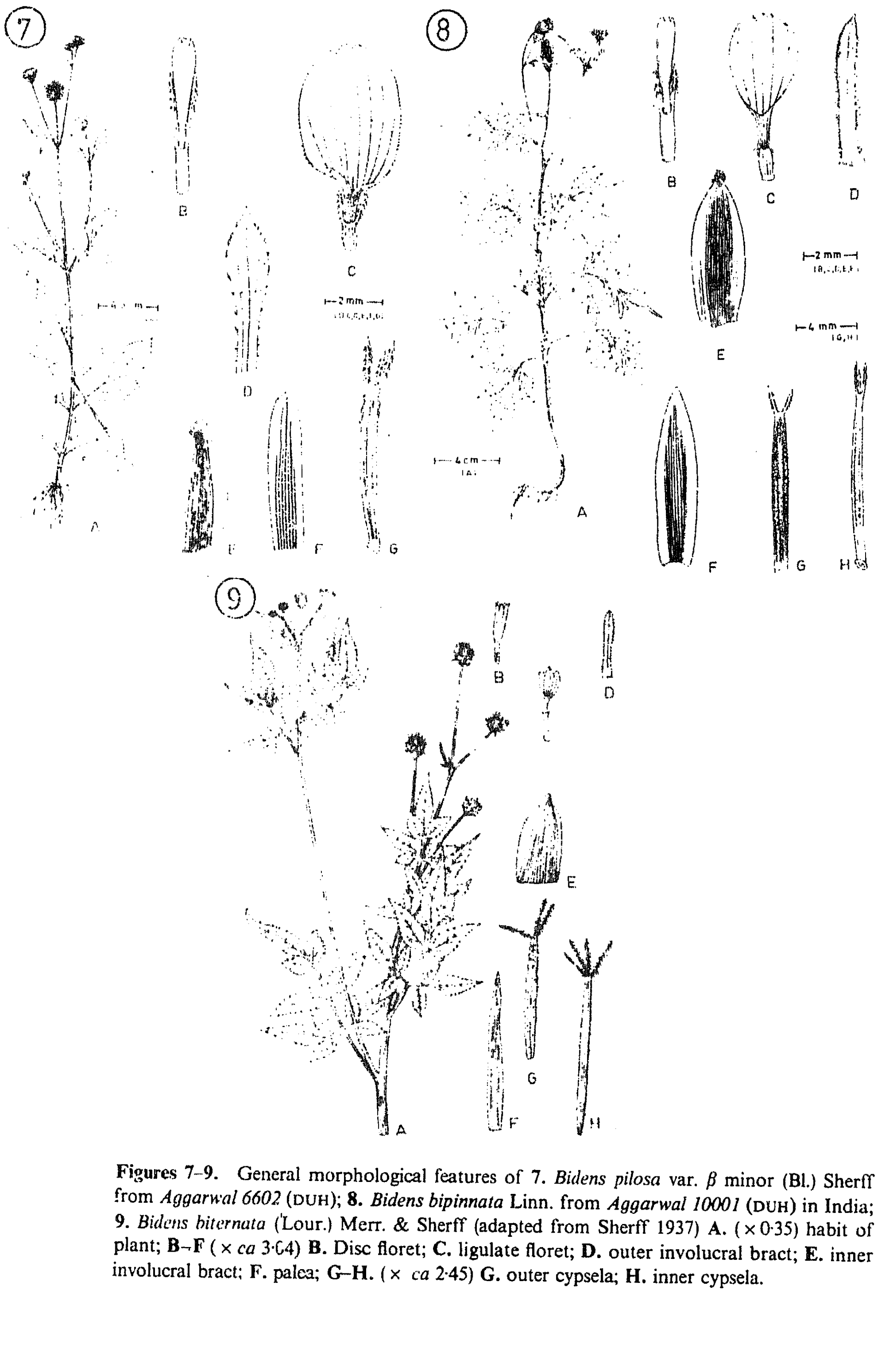
5. Taxonomic enumeration

5.1 *Bidens pilosa var. β minor* (Bl.) Sherff, *Bot. Gaz.* **80** 387 1925 Sherff, *Field Mus. Nat. Hist. Bot.* Vol. XVI, 421–429, 1937. Herbs. Annual to biennial. Erect to decumbent. Decumbent stems when in contact with soil often root at the nodes. Stem 4-angular, longitudinally ribbed; nodes and younger parts glabrous to pilose. Leaf petiolate; lamina entire to pinnatisect into 3 or 5 segments; segments ovate to elliptic-lanceolate, acute to apiculate at apex, margins serrate, serra ciliate, surface, glabrous to pilose. Capitulum heterogamous, radiate, involucral bracts 2-seriate; outer spathulate, connate at the base, pubescent; inner lanceolate, glabrous to pubescent, margin hyaline. Receptacle paleaceous; palea linear-lanceolate, narrower than inner involucral bracts. Ray florets ligulate; ligules white; sterile. Disc florets tubular; yellow; bisexual. Cypselae all of one kind (monomorphic), linear-curved; black; tuberculate-hispid either apically or throughout; with 2 or 3 (rarely 4) retrorsely barbed apical awns (aristae) (figure 7).

Common in moist and shady disturbed habitats, cultivated areas, and gardens. Flowering and fruiting profuse after monsoon. A very widely naturalised weed in warm parts of the world.

Specimens examined

Acland ACK 1307, locality not mentioned, 1926 (BLAT); *Aggarwal 6601, 6602, 6605, 6614, 6615, 6638*, Delhi, 1973 (DUH); *idem 6622, 6625, 15811, 15812*, Nainital, 1975 (DUH); *idem 6644–48* (5 sheets), Kodaikanal, 1975 (DUH); *idem 10604–615* (12 sheets), Kotagiri, 1975 (DUH); *idem 10616–625* (10 sheets), Coonoor, 1975 (DUH); *idem 10626–652* (27 sheets), Bangalore, 1975 (DUH); *idem 10656–658*, Hassan, 1975 (DUH); *anon 15140* (det. J K Maheshwari), Kameng Frontier Division, 1958 (ASSAM); *anon s.n.* (LWG, acc. No. 49052); *Asrava RA 3136*, Dangs, Gujarat, 1956 (BLAT); *Balakrishnan 50132*, Ioski, Khasi & Jaintia Hills, 1969 (ASSAM); *Barnes s.n.*, Bill Hills (DD, acc. No. 10999); *Bhattacharyya 17562*, Mirzapur, 1961 (BSA); *Bole 727*, Girnar Hill, 1952



Figures 7–9. General morphological features of 7. *Bidens pilosa* var. *β* minor (Bl.) Sherff from *Aggarwal 6602* (DUH); **8.** *Bidens bipinnata* Linn. from *Aggarwal 10001* (DUH) in India; **9.** *Bidens biternata* (Lour.) Merr. & Sherff (adapted from Sherff 1937) **A.** (× 0·35) habit of plant; **B–F** (× *ca* 3·04) **B.** Disc floret; **C.** ligulate floret; **D.** outer involucral bract; **E.** inner involucral bract; **F.** palea; **G–H.** (× *ca* 2·45) **G.** outer cypsela; **H.** inner cypsela.

(BLAT); *idem 788, 800*, Saurashtra, 1952 (BLAT); *idem 1444*, North Kanara, 1955 (BLAT); *Bor's collector 142*, Gangtok (Sikkim), 1945 (DD); *Carter 41423*, Shillong, 1915 (CAL); *Dakshini 7234*, Mothronwala Swamp Forest, Dehra Dun (BSD); *idem 13752–755* (4 sheets), Bhopal, 1976 (DUH); *R. N. Dave's collection* (*Santapau 14396*), locality not mentioned, 1951 (BLAT); *Ram Dayal 2556*, Dehra Dun, 1967 (DD); *Deb 30327*, Kottayam District, Kerala, 1968 (MH); *idem 30545*, Lushai Hills, 1963 (ASSAM); *Divakar PD 288*, Karanja, 1959 (BLAT); *idem PD 1093*, Mora, 1960 (BLAT); *idem PD 1262*, Yova, 1960 (BLAT); *Fischer 1207*, Coimbatore, 1906 (CAL); *Henry ANH 181, 1036*, Madras, 1960 (BLAT); *Hooper & Ramaswami 38411*, Tinnevelly District, 1913 (CAL); *Irani NI 5525*, Matheran, 1960 (BLAT); *Lace 1159*, Paunda, 1891 (DD); *Janaki Ammal s.n.*, locality not mentioned, 1955 (BSA); *Jain & Bhardwaja 22597*, Malabar, 1951 (DD); *Kamlesh s.n.*, Meerut, 1959 (LWG, acc. No. 44566); *Maheshwari 122*, Delhi, 1954 (DUH); *Majumdar 10370*, Udaipur, 1966 (BSA); *Mathur s.n.*, Solan, 1938 (DD, acc. No. 78076); *Misra 5333*, Allahabad, 1963 (BSA); *Moonz 1821*, Kardhana, Orissa, 1941 (DD); *Mukerjee 5728*, Tista River, Bengal, 1963 (CAL); *Matthew KMM 1581*, Shembagnur, 1960 (BLAT); *Naithani 3544*, Dehra Dun, 1972 (DD); *idem 1895*, Dehra Dun, 1973 (DD); *Nelson 164 K*, Kurseong, 1914 (DD); *Nilhata N 780*, Madhurai, 1955 (BLAT); *Pandey 219*, Kathmandu (Nepal), 1952 (DD); *Panigrahi 6156*, Kameng Frontier Division, 1957 (ASSAM); *idem 19447*, Subansiri Forest Division, 1959 (ASSAM); *idem 5536*, Kainur Hills, 1962 (BSA); *idem 3091*, Katra (UP), 1964 (BSA); *idem 4047*, Katwa, 1965 (BSA); *idem 12600*, Mirzapur, 1969 (BSA); *idem 12090*, Mirzapur, 1970 (BSA); *idem 15283*, Amarkantak, Bilaspur, M.P., 1972 (BSA); *Panigrahi & Arora 8919*, Bilaspur, 1964 (BSA); *idem 8874*, Bilaspur, 1965 (BSA); *Panigrahi & Prasad 2691*, Pipri (UP), 1964 (BSA); *Patel s.n.*, locality not mentioned, 1882 (BLAT); *Prithipalsingh 2317*, Delhi, 1968 (DUH); *idem 10034, 10047, 10048*, Delhi, 1969 (DUH); *idem 10110, 10111, 10123, 10124*, Bangalore, 1970 (DUH); *idem s.n.*, Hassan, 1970 (DUH); *idem 3342, 3349*, Bangalore, 1971 (DUH); *idem 2742, 2753, 10195*, Delhi, 1971 (DUH); *Raghvendra s.n.*, Delhi, 1957 (DUH); *Rajagopal 6152*, Allahabad, 1964 (BSA); *Raizada 21193*, Junagadh (Saurashtra), 1953 (DD); *Raizada's collectors, Sethi & Negi 26046*, Coimbatore, 1958 (DD); *Randeria AR 550*, Borivli, 1953 (BLAT); *R S Rao 1578*, NEFA, 1955 (ASSAM); *idem 7377*, Kameng Frontier Division, 1959 (ASSAM); *Subba Rao 24779*, Subansiri Frontier Division, 1961 (ASSAM); *idem 29718*, Pottangi (Orissa), 1962 (ASSAM); *Sahni 24138*, Tehri Garhwal, 1955 (DD); *Santapau 7060*, Borivli, 1945 (BLAT); *idem 12665*, Mahbaleshwar, 1951 (BLAT); *idem 16001*, Powai, 1953 (BLAT); *idem 17355*, Dangs, 1953 (BLAT); *idem s.n.* Castle Rock, 1953 (BLAT); *idem 19369*, Dangs, 1954 (BLAT); *idem 20793, 20794, 21509*, Vizag., 1956 (BLAT); *Ram Saran s.n.*, Saharanpur, 1956 (LWG, acc. No. 44526); *Saxena 380, 1057*, Rajpur (Dehra Dun), 1960 (DD); *idem 1275*, Mussoorie, 1960 (DD); *Sebastine 2094*, Nilgiri District, 1957 (MH); *Sengupta 16599*, Raigarh, 1971 (BSA); *Sharma 16815*, Nongpoh, Khasi & Jaintia Hills, 1938 (ASSAM); *Sharma 393*, Nasirabad Ghati (AJMER), 1958 (DD); *S. K. Sharma 15813–15834*, Darjeeling, 1976 (DUH); *Shetty 11905*, Nilgiri District, 1961 (MH); *Schallert 8237*, Florida (USA), 1958 (BLAT); *Shah 4900*, Malad, 1955 (BLAT); *idem 7317, 7318*, Madh Island, 1956 (BLAT); *idem 9116*, Malad 1957 (BLAT); *Shenoy KVS 657*, Mumbra, 1953 (BLAT); *idem 4282*, Mumbra, 1954 (BLAT); *Srinivasan & Singh 62537*, Darjeeling, 1959 (LWG); *Subramanyam 8201*, Madurai District 1959 (MH); *Subramaniam 755*, Vellapatti, 1963 (DD); *Vaid & Thapliyal 25182*, Darjeeling, 1958 (DD); *Verma 43768, 46336, 46675*, North Lakhimpur, Assam, 1960 (ASSAM); *Wagh SKW 2853*, Vizag., 1956 (BLAT); *idem 4704*, Orissa, 1957 (BLAT); *idem 5588*, Tirumalai, Chittoor, 1957 (BLAT); *idem 6378*, Horsely Hills, Chittoor, 1957 (BLAT); *idem 8216*, Tirumalai Hills, Chittoor, 1958 (BLAT).

5.2 *Bidens bipinnata*·Linn. Sp. Pl. 832. 1753; Sherff, *Field Mus. Nat. Hist. Bot.* Vol. XVI, 366–373, 1937.

Erect annual herbs. Stem 4-angular, longitudinally ribbed; nodes sparsely to densely pilose. Leaf petiolate; lamina pinnatisect, 5-, or 7-, or 9- segmented to bipinnatifid; segments ovate-lanceolate to linear, acute, margin serrate, serra ciliate; surfaces pilose (more on the veins). Capitulum heterogamous, radiate. Involucral bracts 2-seriate; outer linear, connate at the base, pubescent; inner ones lanceolate, margin hyaline. Receptacle paleaceous; palea linear-lanceolate, narrower than inner involucral bracts. Ray florets ligulate; ligule yellow; sterile. Disc florets tubular, yellow, bisexual. Cypselae in a capitulum of two kinds (dimorphic) all linear but different in size, colour and surface patterns; outer cypselae generally brown and tuberculate-hispid apically, or blackish-brown and with a hairy (not tuberculate) surface; inner ones black and generally glabrous throughout their length or occasionally very sparsely hairy only in the apical region; with 3 (sometimes 2 or 4 also) retrorsely barbed apical awns (aristae) (figure 8).

The Indian material falls in typical *B. bipinnata* Linn.

Distribution cosmopolitan: in diverse habitats ranging from moist shady areas to dry rocky situations; flowering and fruiting from June to November.

Specimens examined

Aggarwal 6603, 6606, 6613, 6618, 6631, Gurgaon, 1973 (DUH); *idem 6607, 6608, 6617, 6619*, Delhi, 1973 (DHU); *idem 10001, 10002, 10007, 10661*, Gurgaon, 1974 (DHU); *idem 6621, 6623, 6626–6629*, Nainital, 1975 (DUH); *idem 13701, 13705–13749* (47 sheets), Pachmarhi, 1975 (DUH); *anon s.n.*, Poona, 1892 (BLAT, presented by Bombay Natural History Society); *anon s.n.*, Purva, 1912 (LWG, acc. No. 795); *anon 14258*, Bombay, 1917 (BLAT); *Arora 5595*, Mhow, 1964 (BSA); *Bole 412*, Mahbaleshwar, 1951 (BLAT); *Ram Dayal 2556*, Dehra Dun, 1967 (DD); *Garada 21816*, Poona, 1902 (BLAT); *Hooker s.n.*, Sikkim, nodate (CAL); *Irani NI 4604*, Matheran, 1959 (BLAT); *idem NI 5462*, Matheran, 1960 (BLAT); Joseph 11258, Hoshangabad, 1960 (BSA); *Kanjilal 6096* (2 sheets), Barnihat, 1915 (ASSAM, DD); *Mathur s.n.*, Solan, 1938 (DD); *Mazumdar 12495*, Udaipur, 1968 (BSA); *idem 13208*, Kota, 1968 (BSA); *Menon 15801–15804, 6610, 6612, 8821, 8822* (8 sheets), Dalhousie, 1976 (DUH); *Merchant 216*, Matheran, 1957 (BLAT); *Narayanswami 601*, Godavari District, 1920 (CAL); *Neelam 8820*, Mussoori, 1975 (DUH); *Osmaston 1386*, Dehra Dun, 1928 (DD); *Panigrahi 11556*, Kotai Reserve Forest, 1957 (ASSAM); *idem 11800*, Dharampur (Bihar), 1957 (ASSAM); *idem 19695*, Subansiri Forest Division, 1959 (ASSAM); *idem 20652*, Borasamsar (Orissa), 1959 (ASSAM); *idem 6080*, Shivpuri, 1962 (BSA); *idem 4047*, Mirzapur, 1965 (BSA); *Dr Prain's Collector*, *Hock 12*, Naga Hills, 1898 (CAL); *Dr Prain's Collector*, *Naskar 10*, Baidyanath (Bengal), 1902 (CAL); *Prithipalsingh 2497, 7065, 10017, 10018, 10022–10027* (10 sheets), Delhi, 1969 (DUH); *idem 10143, 10144*, Siliserh (Alwar), 1970 (DUH); *R. S. Rao 1349*, N.E.F.A., 1955 (ASSAM); *Santapau 4909*, Salsette, 1944 (BLAT); *idem 5022*, Khandala, 1944 (BLAT); *idem 11183*, Salsette, 1950 (BLAT); *idem 11326*, Purandhar, 1950 (BLAT); *idem 13598*, Rajkot, 1951 (BLAT); *idem 16189*, Saurashtra, 1953 (BLAT); *idem 16667*, Okha, 1953 (BLAT); *idem 19923*, Dangs, 1954 (BLAT); *idem 21397*, Jeypore (Orissa), 1956 (BLAT); *Saxena 1386*, Dehra Dun, 1960 (DD); *S K Sharma 8824*, Simla, 1975 (DUH); *Shenoy KVS 1027*, Matheran, 1953 (BLAT); *idem KVS 4082*, Matheran, 1954 (BLAT); *Tavakari T 1797*,

Goregaon, 1958 (BLAT); *idem T 2033*, Poona, 1958 (BLAT); *Verma 772*, Kishangang (Rajasthan), 1963 (BSA); *idem 46413*, North Lakhimpur (ASSAM); 1966 (ASSAM); *Wadhwa 7428*, Jhalawar (Rajasthan), 1964 (BSA).

5.3 *Bidens biternata* (Lour.) Merr. & Sherff *in* Sherff, *Bot. Gaz.* **88** 293 1929; Sherff, *Field Mus. Nat. Hist. Bot.* Vol. XVI 388–405 1937.

During the present investigation, no specimen could be assigned to this species and consequently no emended description was possible. However, for its identification, it has been keyed on the basis of characters described by Sherff (1937) as well as on the relevant (Sherff 1937) illustration (figure 9). It may also be mentioned that Sherff (*loc. cit.*) listed 17 specimens of this species collected from India, but all these specimens are located in herbaria outside India and these could not be examined.

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The authors are indebted to Prof V H Heywood (University of Reading, UK) and to Mr B L Burtt (Royal Botanic Garden, Edinburgh, UK) for discussion on the taxonomy of the genus *Bidens* and for their valuable comments. Thanks are extended to Dr C R Babu for helpful suggestions. Their sincere thanks to the Officers of the Botanical Survey of India, Forest Research Institute, National Botanical Research Institute, and the Blatter Herbarium (St. Xavier's College, Bombay), for the loan of herbarium specimens for study. The supply of photographs of authentic specimens from the *Kew, Gray, Field Museum of Natural History*, and *British Museum* (*Natural History*) herbaria is gratefully acknowledged.

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Free amino acids in the developing leaves and flower bud of *Abelmoschus esculentus* (L.) Moench

E NABEESA and N NEELAKANDAN

Department of Postgraduate Studies and Research in Botany, University of Calicut, Calicut 673 635, India

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Abstract. Free amino acids were determined at six chronologically comparable developmental stages each of leaves 1 and 2, bearing vegetative dormant axillary meristem and leaves 3 and 4, bearing axillary flower bud, in *Abelmoschus esculentus*. Simultaneously, free amino acids were determined in the flower bud at the third leaf axil at four stages of growth. The developmental pattern of the amino acids has been interpreted in the light of the source activity of the leaves.

Keywords. *Abelmoschus esculentus*; aging; axillant leaf; axillary flower bud; free amino acids; sink; source.

1. Introduction

All proteins are not elaborated or degraded at the same time or at the same rate in the green leaves (Huffaker and Peterson 1974). The elaboration of tissue-specific and organ-specific proteins (Zacharias *et al* 1957; Barber and Steward 1968; Pierard *et al* 1980) and the preferential degradation of the proteins of nonessential floral parts (Cutter 1971) may be expected to bring about alterations in the free amino acid spectrum of the flower bud during development. Since the developing flower bud is largely dependent on the axillant leaf (Ashley 1972), changes in the protein composition and amino acid distribution in flower bud will be reflected in the amino acids in the leaf. It is to be expected that the spectrum of free amino acids in a leaf will vary with its position on the plant, type of axillary tissue and developmental stage of the leaf and subtending tissue. The early literature on the free amino acids in attached leaves was reviewed by McKee (1958). Ontogenetic and age-dependent changes in free amino acids content of wheat leaves were studied by Weinberger (1975). Housley *et al* (1979) reported free amino acid changes in soybean leaves during the flowering process.

In the present investigation, a comparative study has been made of the developmental distribution of free amino acids in four successive leaves of *Abelmoschus esculentus*, two of which carried vegetative, dormant, axillary buds and, the other two, active reproductive axillary buds. The distribution of free amino acids in the developing flower bud at the axil of one of the leaves was simultaneously studied. The results obtained permitted conclusions to be drawn about the ontogenetic and age-dependent/age-related changes in free amino acids in leaves as influenced by the type of axillary growth and the relation between free amino acids in leaf and subtended flower bud.

2. Materials and methods

2.1 *Plant material*

Abelmoschus esculentus (L.) Moench, variety 'Pusa-sawani', was used in the study. A distinctive feature of the variety is that the reproductive bud is borne at the axil of the third (true) leaf upward and the reproductive meristem is present when the axillant leaf is a primordium. The plants were raised in garden soil in pots under natural environmental conditions. The first (L1) and second (L2) leaves which bore dormant vegetative bud and the third (L3) and fourth (L4) leaves which bore reproductive bud in their axils were analysed. Commencing from L4, there was increasing invagination of the leaf margin.

2.2 *Sampling*

The first sample of every leaf was collected on the day it unfolded. The timing of sample collection was fixed at 10·00 hr. With a sufficiently large number of plants available, it was possible to choose leaves which had unfolded shortly before 10·00 hr. Six samples each of the four leaves were collected. Successive samples of a leaf were collected as successive leaves on the plant unfolded, which occurred every 3–4 days. When L2 unfolded, the second sample of L1 was collected along with the first sample of L2. When L3 unfolded, the third sample of L1 and the second sample of L2 were collected along with the first sample of L3. This pattern was continued until with the unfolding of the 9th leaf, the final sample of L4 was collected. Flower bud formed in the axil of L3 was collected at four developmental stages, starting from floral organogenesis (Fl_1) and ending with preanthesis (Fl_4) and coinciding with the collection respectively of L3 samples 1 to 4. Separate histological studies (unpublished) showed that in Fl_1, the sepals and petals differentiated and that the androecium was in the process of differentiation; in Fl_2, the gynoecium also started differentiating; in Fl_3, the first mitotic division of microspores occurred and the gynoecium was in the advanced state of differentiation. Fl_4 was the preanthesis stage of the flower bud; the embryo sac differentiated and the pollen grains were fully mature.

2.3 *Analytical methods*

Free amino acids were extracted in the hot with 80% (v/v) ethanol and purified with Dowex 50 W-x8 according to Kliewer (1964). The amino acids were separated by two-dimensional descending paper chromatography using *n* butanol : acetic acid : water (100 : 22 : 50) and estimated according to Giri *et al* (1952). Cysteine was detected and estimated as cystine. Isoleucine and lucine were not clearly separated from each other on the chromatogram and were determined as a pair; for a similar reason, methionine and valine were determined together. All determinations were carried out a minimum of 5 times to permit statistical evaluation.

3. Results

3.1 *Leaf*

Figures (1–4) show the distribution of free amino acids in leaves. In L1, glycine was the most abundant amino acid in the first sample, followed by glutamic acid. Aspartic acid,

serine, threonine and cysteine were present in comparatively large amounts. Proline and hydroxyproline could not be detected (in any of the samples of L1). In the second sample, alanine was most abundant, closely followed by threonine. Aspartic acid, glutamic acid, glycine and serine decreased considerably and γ-aminobutyric acid increased. Asparagine, low in the first sample, was reduced to traces in the second sample (and could not be detected in subsequent samples). Cystine, high in the first sample, was in traces in the second sample (and all the succeeding samples). In the third sample, threonine was most abundant, followed by alanine. In the fourth sample, γ-aminobutyric acid became the most abundant followed by threonine. Samples 5 and 6 of L1 showed a marked decrease in many amino acids; also, fewer amino acids were present than in the earlier samples. γ-Aminobutyric acid, glutamic acid and glycine were maintained at comparatively high level in sample 5 and alanine and γ-aminobutyric

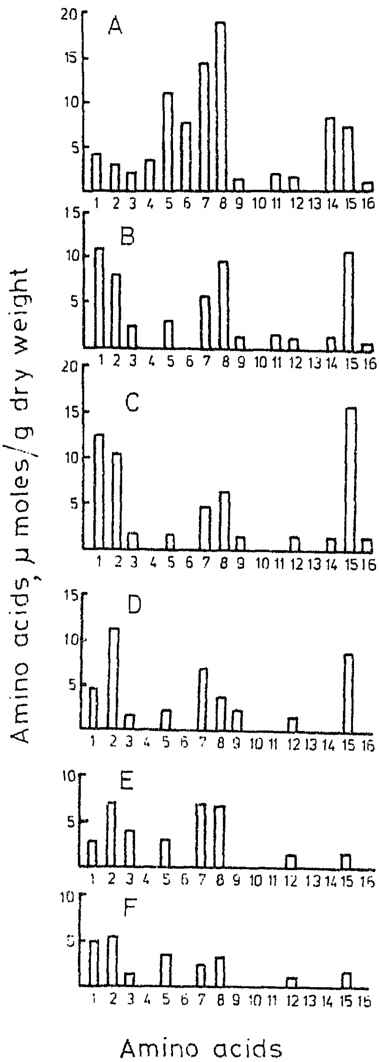


Figure 1.

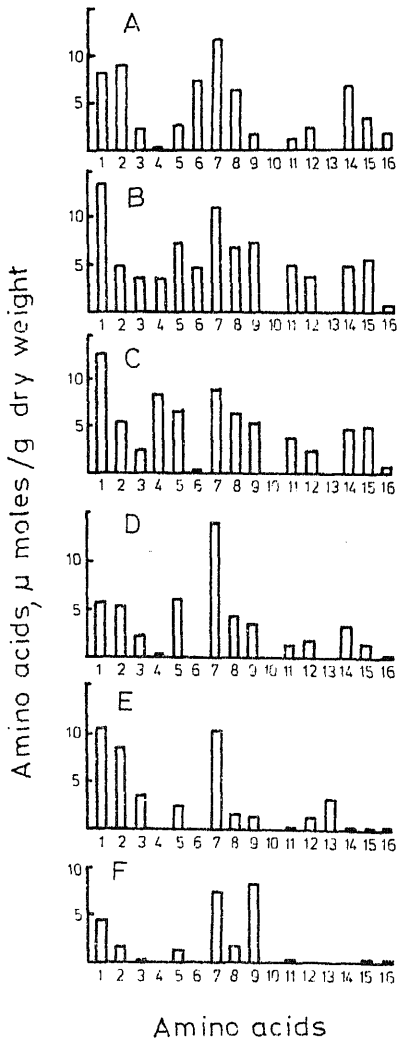
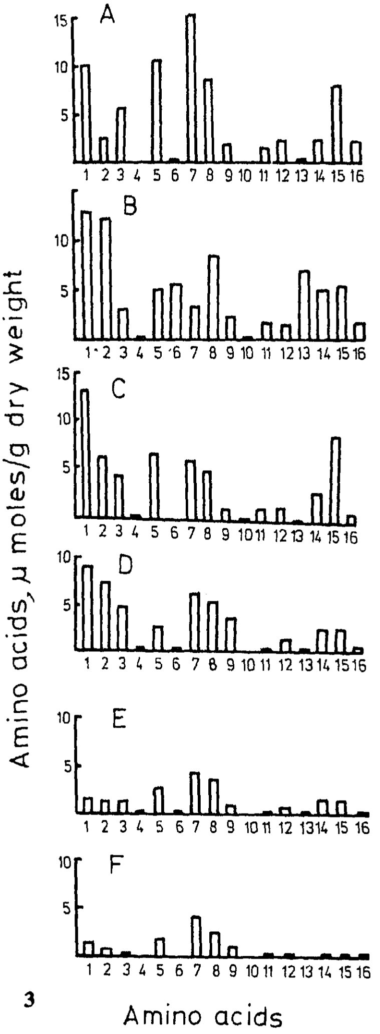


Figure 2.



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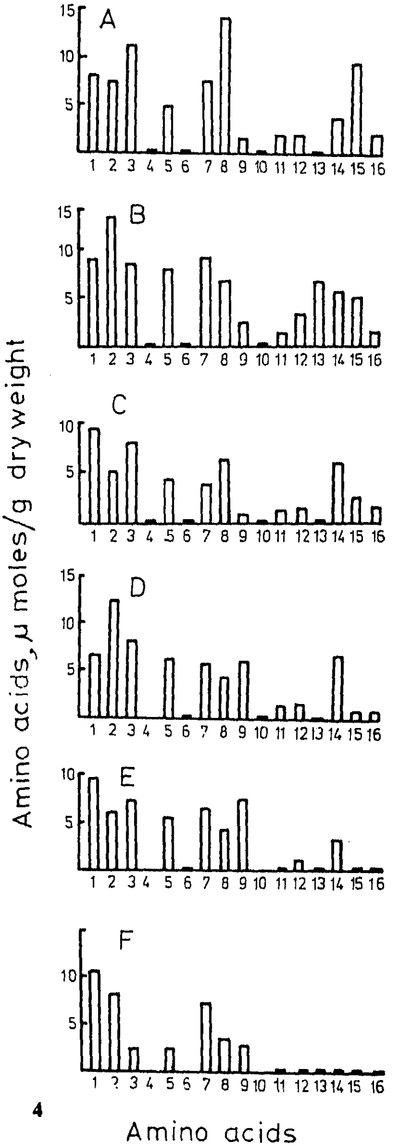

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Figures 1–4. Concentration of free amino acids during 6 growth stages of four leaves. **1.** L1, **2.** L2, **3.** L3, **4.** L4. (Numbers 1–16 denote the individual amino acids and letters A-F the sample number). 1. alanine, 2. γ-aminobutyric acid, 3. arginine, 4. asparagine, 5. aspartic acid, 6. cystine, 7. glutamic acid, 8. glycine, 9. histidine, 10. hydroxyproline, 11. leucine + isoleucine, 12. valine + methionine, 13. proline, 14. serine, 15. threonine, 16. tryptophan. (**A.** sample 1, **B.** sample 2, **C.** sample 3, **D.** sample 4, **E.** sample 5, **F.** sample 6).

acid in sample 6. The highest arginine concentration in L1 was in the 5th sample.

In L2, glutamic acid was most abundant in the first sample, followed by γ-aminobutyric acid. Aspartic acid and glycine were comparatively low and alanine was high in concentration. Asparagine was in traces. Alanine became the most abundant amino acid, followed by glutamic acid, in the second sample; asparagine, histidine and the leucines increased. Alanine was the predominant amino acid, followed by glutamic acid, also in sample 3. Asparagine, whose concentration was more than doubled, now became the third in abundance. Cystine was reduced to trace level (to remain at this level or to disappear in the subsequent samples). In the fourth sample, alanine decreased and glutamic acid became the most abundant, with aspartic acid a distant second. Asparagine was reduced to traces (to disappear in later samples). In sample 5, alanine was once again the most abundant, to be closely followed by glutamic acid. γ-Aminobutyric acid increased in concentration. In the final sample, histidine, which had increased several-fold, occurred in the highest concentration; which was followed by glutamic acid. The number of amino acids present in assayable amounts was reduced to six in sample number 6. As in L1, proline, was not detected in any sample of L2.

In L3, as in L2, glutamic acid was the most abundant amino acid in sample 1; this was followed by aspartic acid and alanine. Threonine and glycine also occurred in high concentration; serine concentration was comparatively low. Among the various samples from all leaves, the highest tryptophan concentration was found in sample 1 of L3. Asparagine could not be detected; as a matter of fact, in no sample of L3 was asparagine present in assayable amounts. In the second sample, alanine was the most abundant, with γ-aminobutyric acid a close second. Cysteine occurred in fair amounts, which contrasted with its occurrence in traces only, or not detected at all, in all the other samples of L3. Glutamic acid, aspartic acid and threonine decreased. Proline, which was in traces only in the first sample, was in high concentration in the second sample (to reduce to trace levels again or to disappear in subsequent samples). In the third sample of L3, alanine, unchanged in concentration, became the most abundant amino acid. Threonine, which increased in concentration, became the second most abundant. Glutamic acid and aspartic acid also increased in concentration. In the fourth sample, alanine was again the most abundant, with γ-aminobutyric acid as the second. Glutamic acid and glycine were in high concentration. Glutamic acid was the most abundant and glycine the second in samples 5 and 6. Only six amino acids were present in assayable amounts in the final sample.

In L4, as in L1, glycine among all the amino acids occurred in the highest concentration in the first sample. Arginine was the second highest. Threonine, alanine, glutamic acid and γ-aminobutyric acid occurred in high concentration. Asparagine was in traces, the succeeding samples also containing only traces, if at all, of the acid amide. Cysteine occurred in trace only, a feature which persisted till the fifth sample, to disappear altogether in the final sample. In the second sample, γ-aminobutyric acid was the most abundant and glutamic acid/alanine the second. Arginine concentration was reduced, but it was still a major amino acid. Proline, which was in trace in sample 1, (as also in samples 3 to 6) occurred in high concentration in sample 2, though without simultaneous reduction in glutamic acid. Alanine was the most abundant in the third sample and γ-aminobutyric acid in the fourth; arginine was the second most abundant in both samples. Histidine concentration markedly increased in sample 4. Glutamic acid was the most abundant in sample 5, with alanine a close second. Arginine continued to be maintained in high concentration. Histidine also was in high

concentration, as in sample 4. Alanine was the most abundant and γ-aminobutyric acid the second most abundant in the final sample. Glutamic acid continued to be in high concentration. In all, seven amino acids were present in assayable amounts, the others being in traces or not detected at all.

3.2 *Flower bud*

Arginine, in high concentration in the first sample, showed a progressive increase with development, becoming the most abundant amino acid from stage 2 onward and being present in over 4-fold higher concentration in the final as in the first sample (figure 5). Alanine was the most abundant among the amino acids in the first sample and the second most abundant in the third and fourth samples; the concentration decreased in the second sample. Asparagine was low in concentration in the first and second samples, but it was a major component in the third and fourth samples. Cysteine was present only in traces in the first and second sample, but the third and fourth samples contained high concentration of the amino acid. γ-Aminobutyric acid was the second most abundant amino acid in the first sample, but it was in comparatively low concentration in the subsequent samples. Glutamic acid, aspartic acid, serine, glycine and threonine, which occurred in high concentrations in many leaf samples, were

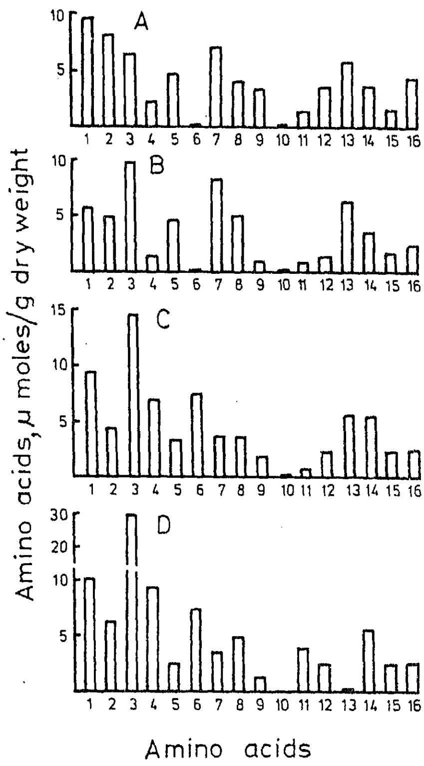

Figure 5. Concentration of free amino acids during 4 growth stages of flower bud at L3 axil. Other details are as under figures 1-4.

present only in moderate concentrations in the bud samples. Tryptophan was in comparatively high concentration in the first sample and was maintained in appreciable concentration in the following samples contrasting with the low concentration in which it occurred in leaves except in the first sample of L3. Samples 1, 2 and 3 contained substantial amounts of proline; but there was abrupt decrease to trace level in sample 4.

4. Discussion

While interpreting the findings, a few generally accepted physiological concepts have to be kept in view; (i) A leaf inserted at the top will be the preferred source for the shoot apex. (ii) A leaf located lowest on the stem will be the preferred source for the root system. (iii) For a developing axillary flower bud and fruit, the axillant leaf will be the primary source (Ashley 1972). In view of the powerful sink activity of the flower bud and, more so, the fruit, a leaf in the immediate neighbourhood may also function as a source supplementing the axillant leaf (Bhatt 1976). (iv) Leaves inserted lower down on the stem will have easier access to metabolites elaborated in the roots and transmitted in the xylem stream (Pate 1980). (v) Amino acid synthesis is not confined to leaves, but occurs in other tissues as well and transfer among tissues can occur. Roots are capable of synthesizing a number of amino acids. (vi) It is believed that the transition from a juvenile to adult form of leaf (heteroblasty) may be associated with transcriptional changes (Nagl 1979).

The failure to detect lysine, phenylalanine and tyrosine may be due to insufficient concentration. In view of the central role played by glutamine, a role which cannot apparently be taken over by asparagine, it has to be inferred that glutamine did occur, but in amounts too low to be detected.

Photosynthetic production of carbohydrates is coupled to amino acid formation (Housley *et al* 1979). Since whole extracts of leaves were analysed, conclusions could be drawn about the net pool of amino acids, but not of amino acids produced during current photosynthesis. The data for free amino acids gave no clear indication that changes in their distribution pattern conformed to definite families of amino acids, or to intrafamilial transformations. In no case did the composition of free amino acids as in leaf correspond to that of the bound amino acids in fraction I protein (Wildman and Jagendorf 1952).

The four bud samples showed broad resemblances, with some differences, among themselves, in the distribution of amino acids. The pattern of amino acid distribution in the buds did not conform to that of any leaf, inclusive of the axillant leaf (figures 1–5). Expressed in terms of the dry solids in tissues, several of the amino acids occurred in higher concentration in the flower bud than in the axillant leaf, suggesting transport from leaf against a concentration gradient. It is known that the total free amino acids and amides are usually higher in pollen than in leaves or other tissues (Stanley and Linskens 1974). The green tissues of the epicalyx and calyx in stages 1 and 2 might elaborate amino acids by coupling their synthesis to carbohydrate biosynthesis by photosynthetic fixation of carbondioxide. Secondary changes in amino acids such as by amino transfer reactions could not be ruled out.

Glycine, serine, alanine and threonine occurred in high concentration in leaves at certain stages and underwent marked fluctuations in concentration at other stages. The occurrence of γ-aminobutyrate in high concentration in many of the leaf samples was in

conformity with the wide distribution of this non-protein amino acid in plants. It is believed that asparagine and arginine act as temporary stores of reduced nitrogen, particularly under conditions of carbon stress and nitrogen excess (Miflin and Lea 1977). Once synthesised, the catabolism of asparagine appears to be comparatively slow. Asparagine occurs in traces, or not at all, in L3 and L4; it was, however, present in readily assayable amounts in certain stages of L1 and L2. The proximity to the root system will probably account for the presence of asparagine in L1 and L2. The abrupt decrease in asparagine which occurred in L1 and L2 at some stages signified utilization or export. The failure of asparagine to accumulate in L3 and L4 may be due to transfer to the flower bud; asparagine was the third most abundant amino acid in the pre-anthesis bud. During leaf senescence amino acids are increasingly utilized as respiratory substrates. The nitrogen split off in this process accumulates as asparagine in the detached leaf (Chibnall 1939). There was no indication of asparagine accumulation in the attached fifth and sixth sample of any leaf, inclusive of L1 which showed visible senescence. Arginine was a minor component in L1 and L2 and to an extent also in L3. In L4, it was a major component. The flower bud at L3 axil had, however, high concentration of arginine. Cysteine occurred in appreciable amounts in the second sample of L3, but the amino acid was in traces only, or not detected at all, in L4 samples.

Special interest centred round proline. Proline is one of the most abundant free amino acids in angiosperm pollen, constituting 1 to 2.2% of dry weight (Stanley and Linskens 1974). In the present studies, assayable amounts of proline were present only in L3 and L4 and that too only in the second sample of each, which corresponded to the second sample of flower bud at L3 axil and its equivalent at L4 axil. However, proline occurred in substantial amounts also in the first and third samples of the flower bud when the axillant leaf contained only traces. These data suggested that proline in flower bud might not represent the amino acid translocated from leaf, but was likely to have been formed *de novo* in the tissues of the bud. The reason was not clear for the presence, only of traces, of proline in the fourth sample of flower bud when the pollen grains were fully mature and anthesis was imminent.

The flower bud produces relatively large amounts of auxin. The shoot apex meristem also is an important site for auxin production. Tryptophan, the precursor of auxin, was present in leaves and flower bud, the concentration in the latter exceeding that in the former.

Apart from the quantitative differences pointed out above, a major distinction in amino acid distribution between L1 and L2, on the one hand and L3 and L4, on the other, was not apparent. L4 differed from L3 in some respects. Whether the quantitative distinctions among the leaves was related to the type of axillary meristem, or to ontogeny, or to heteroblasty, or to a combination of these factors, was not certain.

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Factors controlling growth rate of cellulolytic fungi on sterile filter-paper

S D GARRETT

University of Cambridge, Botany School, Downing St., Cambridge CB2 3EA, England

Abstract. Cellulolysis rates of four cereal foot-rot fungi were estimated by dry-weight loss (WL) of filter-paper cultures incubated for 7 weeks at 22·5°C. Fungal growth rate across the paper circles was recorded and was found to be correlated with the product of $\sqrt{\text{WL}}$ of paper multiplied by fungal growth rate over PD agar; the correlation was significant at 1% level.

Keywords. Cellulolysis; cereal foot-rot fungi.

1. Introduction

In introducing this paper, I must first say how much I have appreciated the honour of being invited to contribute something to the Golden Jubilee of the Indian Academy of Sciences. The name of its famous Founder and first President, Sir C V Raman, has long been familiar to me, and to most other biologists as well as to all physicists. So I was immensely pleased by my election as an Honorary Fellow of the Academy eleven years ago; never before had I found myself in the company, at least on paper, of so many Nobel Laureates. The Academy's second President, Professor T S Sadasivan, has been a friend of mine for some forty-five years, ever since the days when we worked together as young men at the Rothamsted Experimental Station. His paper of 1939 reported the discovery that *Fusarium culmorum*, which is a fungus causing a seedling blight and foot-rot of cereals, is also a widespread colonizer of wheat straw buried in soil, as when straw is ploughed under the soil after harvest.

When I moved to Cambridge in 1949, Sadasivan's work with *F. culmorum* was one of the foundations for a method that we devised for quantitatively assessing the competitive saprophytic ability of cereal foot-rot fungi for colonization of wheat straw buried in soil, in competition with obligate saprophytic soil fungi; *F. culmorum* was found to possess a high degree of competitive saprophytic ability, whereas that of the take-all fungus, *Gaeumannomyces graminis* var. *tritici*, was found to be comparatively low (Butler 1953; Lucas 1955). Later Macer (1961), working with five species of cereal foot-rot fungi, found that success in competitive saprophytic colonization of straw was closely correlated with speed of fungal penetration of the straw tissue from the outside. Wheat straw contains *ca* 40% cellulose, which is a major structural component of the tissue and a major carbon substrate for cellulolytic soil fungi. So I developed a method for estimating the cellulolysis rate of cereal foot-rot fungi, by determining loss in dry weight of filter-paper cultures incubated for a standard period of 7 weeks at 22·5°C (Garrett 1966). After making a number of such determinations of cellulolysis rate, I found that, for five fungi, cellulolysis rate was closely correlated with straw-penetration rate, as determined by Macer (Garrett 1975). This led me to the final conclusion that

success in competitive saprophytic colonization of wheat straw by cereal foot-rot fungi was chiefly determined by their cellulolysis rate. This realization then led me to ascertain whether saprophytic survival of such a fungus was also affected by its cellulolysis rate; I found that, for five isolates of *Cochliobolus sativus*, the median survival period (the S_{50} value) in colonized straw buried in soil was correlated with the cellulolysis rate of the fungal isolate (Garrett 1978).

In this study I recorded growth rate across filter-paper circles and over potato-dextrose (PD) agar by eight isolates of *C. sativus* for which these data were available. A correlation was found between growth rate over paper and cellulolysis rate. The value of the correlation coefficient (r) was 0·8886, which for $n = 7$ is significant at the 1 % level. The regression of fungal growth rate (x) on cellulolysis rate (y) is given by the equation $x = 0{\cdot}405\ y + 0{\cdot}408$; the regression line for this equation has been plotted in figure 1, which has been reproduced from Garrett (1978).

No correlation was found between fungal growth rate across paper and that over PD agar. My recent work, to be described below, has questioned this conclusion, which I am now able to explain. Some of the isolates employed in this study declined in growth rate after a short period of colony growth on PD agar, due to the accumulation of fungistatic growth-products and so PD agar did not afford a substrate suitable for measuring maximum intrinsic radial growth rate; it now seems likely that this was much the same for seven of the isolates, at *ca* 6 mm 24 hr^{-1}. The remaining isolate (*C*) was a laboratory saltant with exceptionally low growth rate and cellulolysis rate, values for both of which are expressed by the lowest point on the regression line in figure 1.

The second error that I made in interpreting these results lay in my failure to realize at that time that we should not expect radial growth rate of a fungal colony across the paper circle, to be *directly* correlated with weight loss (WL) of the paper (as a measure of fungal cellulolysis rate). Weight loss must vary with the area (πr^2) of the fungal colony and not with its radius (r). So if WL varies with τ^2, then r must vary with $\sqrt{\text{WL}}$. To test this conclusion, I have now determined the degree of correlation between growth rate of these isolates of *C. sativus* across paper and their values for $\sqrt{\text{WL}}$; the value of the correlation coefficient is 0·9192, which is higher than that for the relationship shown in figure 1, and is significant at the higher probability level of 0·1 %. The regression of fungal growth rate over paper (x) on $\sqrt{\text{WL}}$ (y) has been calculated, and is expressed by the equation $x = 1{\cdot}7\ y - 1{\cdot}17$. The regression line for this equation has been plotted for figure 2.

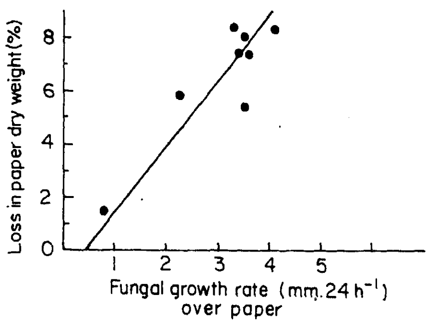

Figure 1. Regression of fungal growth rate on percentage loss in dry weight of paper (reproduced from Garrett 1978).

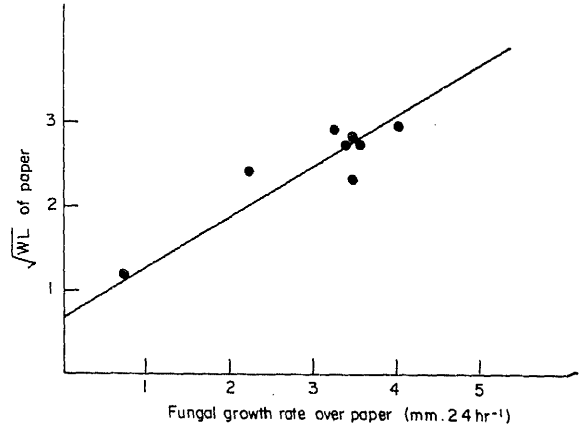

Figure 2. Regression of fungal growth rate on square root of percentage loss in dry weight of paper.

Figure 2 shows that the eight points lie more closely along the regression line than they do in figure 1. This is compatible with the supposition that fungal growth rate across the paper is related to $\sqrt{\text{WL}}$ rather than to WL of paper. The experiment now to be described was designed to confirm this conclusion for *C. sativus* and for three other species of cereal foot-rot fungi. The isolate of *C. sativus* was selected as one that did not stale on PD agar, so that for this and the other three fungal isolates, growth on PD agar gave a reliable estimate of maximum growth rate of each species. This would make it possible to ascertain whether fungal growth rate across paper is affected by differences in radial growth rate over PD agar characteristic of the four fungal species.

2. Materials and methods

Isolates of the following cereal foot-rot fungi were employed in this experiment: *Fusarium culmorum* (W. G. Sm.) Sacc.; *Cochliobolus sativus* (Ito and Kuribay.) Drechsl. ex Dastur; *Curvularia ramosa* (Bain.) Boedijn; *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *tritici* Walker. Methods for measuring fungal growth rate over filter-paper circles and for determining dry-weight loss of paper, as a measure of cellulolysis rate, have been fully described in Garrett (1978), so only a summary will be needed here. Twenty conical flasks (250 ml) each received a wad of ten Whatman no. 3 filter-papers (7 cm diam.), which were weighed air-dry and then moistened with 16·5 ml of a mineral nutrient solution; the resulting moisture content of the paper wads was then 82·5 % of saturation. After autoclaving for 30 min at 121°C, one replicate series of four flasks was inoculated with each fungus: the fifth series was left until the end of the experiment, when its paper wads were oven-dried and weighed; from this, a factor for converting original air-dry weights of the fungus-inoculated paper wads to over-dry weights was obtained. The wad of paper in each fungal series of four flasks was inoculated at the margin of the wad, touching the glass wall of the flask, with one inoculum disk (10 mm diam.) taken from the growing margin of a fungal colony on PD agar. Flasks were inspected daily, and the number of days taken by each fungal colony to cross the 6 cm

Table 1. Growth rate of fungal colonies over paper and weight losses caused by them.

	a	b	c	d	e
	Mg wt. lost by paper (WL)	$\sqrt{\text{WL}}$	Growth rate over PD agar (cm. 24 hr^{-1})	$b \times c$	Growth rate over sterile paper (mm. 24 hr^{-1})
F. culmorum	16·4	4·05	1·03	4·17	3·75
G. sativus	22·3	4·72	0·65	3·07	2·38
C. ramosa	21·4	4·63	0·50	2·32	1·68
G. graminis	4·4	2·10	0·67	1·41	1·25

SE for loss in paper dry-weight = 1·878; for fungal growth rate over paper = 0·4117; LSD for loss in wt. = 4·25 mg; for growth rate = 1·32 mm.

distance between the inner edge of the inoculum disk and the opposite margin of the paper circles was recorded. From this, growth rate of each fungal colony could be calculated, and expressed as mm 24 hr^{-1}. At the end of the experiment, after 48 days' incubation at 22·5°C, paper wads were oven-dried to constant weight at 80°C, allowed to cool down to ambient temperature in a desiccator and then weighed to the nearest mg. In expressing the results of these weighings in table 1, mean loss in oven-dry weight (mg) for each fungal series refers to loss by a single paper circle.

3. Results and discussion

The data in table 1 show that growth rate of the four fungal species across paper cannot be directly correlated either with their cellulolysis rates, as expressed by weight loss (WL) of papers, or with their intrinsic radial growth rates, as expressed at its maximum on PD agar, in numerically convenient units of cm. 24 hr^{-1}. But the data do suggest that growth rate across paper may be controlled by a combination of these two factors, cellulolysis rate and intrinsic radial growth rate, acting jointly. Trials have shown that the closest fit to a straight line is given by plotting fungal growth rate across paper against the product of square root of weight loss by paper ($\sqrt{\text{WL}}$) multiplied by fungal growth rate over PD agar. Testing for the significance of this correlation, we find that the value of the correlation coefficient (r) = 0·9827, which, for n = 3, is significant at the 1 % level. Calculating the regression of x (growth rate across paper) on y (product of $\sqrt{\text{WL}}$ × growth rate over PD agar), we get: $x = 0{\cdot}92\,y - 0{\cdot}25$. The regression line for this equation is shown in figure 3.

Figure 3 shows that the four points given by the paired values of x and y lie close enough to the regression line to be consistent with experimental error. This confirms my revised conclusions from my earlier study of eight isolates of *Cochliobolus sativus*: firstly, that cellulolysis rate varies as the square root of weight loss ($\sqrt{\text{WL}}$) by fungal cultures on papers; secondly, that real differences in intrinsic growth rate between fungal isolates, as expressed by growth over PD agar, will also be reflected by differences in growth rate across paper.

In conclusion, it is instructive to compare growth rates of these four fungal isolates

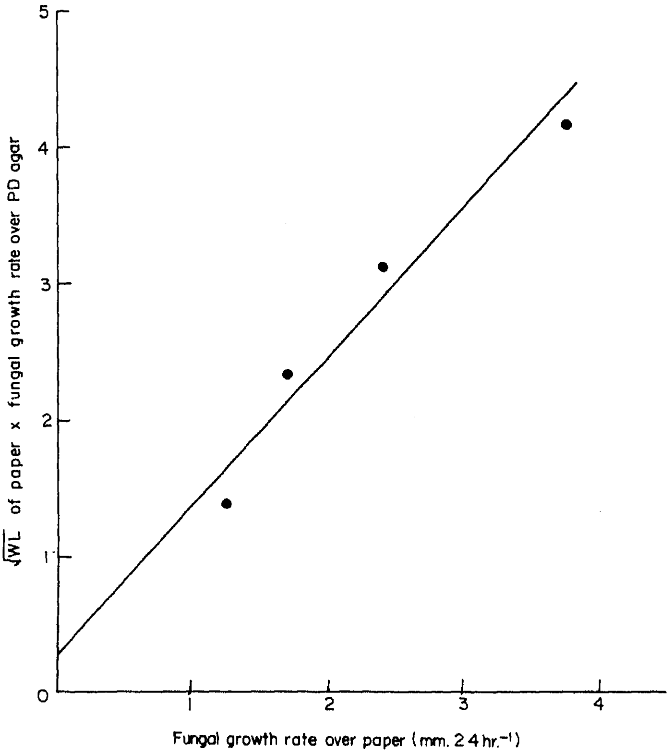

Figure 3. Regression of fungal growth rate over paper on $\sqrt{WL}$ of paper × fungal growth rate on PD agar.

ıcross sterile paper, as shown in table 1, with those of the same isolates over *unsterilized* paper in the presence of commensal bacteria (table 1, Garrett 1983). The comparison ıas been made with figures for growth over unsterilized paper at a similar moisture ontent of 75 % saturation. On unsterilized paper, fungal growth rates were reduced by acterial competition which presumably was chiefly for the sugars released by fungal ellulolysis. Growth over unsterilized paper was reduced to the following percentages f that on sterile paper: *F. culmorum*, 11; *C. sativus*, 13; *C. ramosa*, 12; *G. graminis*, 8. 'hese figures are of the same order of magnitude, and suggest that the four fungi do not iffer significantly in their tolerance of bacterial competition. This conclusion would ot have been possible without the work described above and will, I hope, make the ubject of wider interest.

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The many types of disease resistance

R K S WOOD

Department of Pure and Applied Biology, Imperial College, London SW7 2BB, UK

Keywords. Disease resistance; parasite; induced resistance; race-specific; race non-specific; non-host resistance.

This paper deals with diseases caused by microorganisms as parasites when they grow inside higher plants. For a particular plant the extent to which a parasite does not grow and, therefore, cause disease is a measure of disease resistance. The causes of resistance are the conditions in the plant that limit growth. They are of two main types. The condition may be independent of the parasite as when cell walls of uninfected plants are lignified and therefore potentially resistant to the penetration and degradation upon which growth of the parasite in the plant may depend. Or the condition may be a response to the parasite so that, for example, unlignified cell walls become lignified and again impede growth of a parasite. This second induced type of resistance is almost certainly much more important and is therefore the subject of this paper. It will be discussed under three main headings for which the least unsatisfactory terms probably are race-specific, race non-specific and non-host resistance.

1. Race-specific resistance

In this, by far the most studied, cultivars of a plant are differentially resistant to races of a parasite in patterns which in some diseases have been shown to reflect a matching of a host gene for resistance (R) and a pathogen gene for low virulence (P) for a series of pairs of such genes.

Resistance almost always is dominant and in the few diseases in which genetic analysis of the pathogens has been possible, avirulence is dominant to virulence. When a pair of matching genes ($P \times R$) which would confer resistance occurs with another ($p \times r$) which alone would mean susceptibility, then the phenotype is resistant. From these and related facts it may be inferred that it is $P \times R$ that is determinative and that when it does not occur ($p \times R$, $P \times r$, $p \times r$) then the phenotype is susceptibility. This inference is the basis of most past and current research on resistance. Its aim has been to determine how a low virulence gene P matched by a resistance gene R leads to conditions which limit growth of the parasite. As might be expected it has been usual to select for study diseases in which $P \times R$ allows very little growth of the parasite and little disease whereas the other combinations allow much more of both. The highly resistant reaction is usually associated with death of one cell or of a few cells to which the parasite is confined. It is usually referred to as a hypersensitive reaction or response (HR). Whether or not the parasite remains alive in the killed cells has rarely been determined but its death is not critical in the context of resistance. Neither need be the death of host cells which is usually regarded as an essential feature of HR. Changes in host cells falling short of death could be and almost certainly are in some diseases every bit as effective in

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severely limiting growth of a parasite. The essential feature of HR is perhaps not so much the nature and expression of the changes in cells containing or close to the parasite as the effect of these changes in drastically restricting its growth. And even thus it must be remembered that in some diseases and for different pairs of $P \times R$ genes there is a continuum between HR as high resistance and the parasite confined to one cell or a few cells and intermediate resistance (or low susceptibility) involving many more cells not all of which are killed. Plant pathologists have perhaps become too preoccupied with death of cells as a criterion of HR. Some have gone so far as to describe the killing of cells by an abiotic agent as HR. This would possibly be justified if the agent were also the agent from a parasite which caused HR or if it acted through the same mechanism. But any less discriminating use is hardly justified and can be confusing.

Next and briefly the conditions that restrict growth and first of the obligate parasite which continues to grow only in association with host cells that remain alive although, of course, almost certainly different and possibly very different from corresponding cells in uninfected tissues. Death of cells no matter how caused could explain resistance but from this it must not be assumed that it does. The parasite may cause other changes which prevent growth of the obligate as they do of the facultative parasite and these may or may not depend upon cell death.

Death of cells almost always will not explain resistance to facultative parasites which can grow on dead cells. Neither would damage short of death because probably this would release substances that would be expected to sustain the growth of a parasite. Therefore what mechanisms of resistance are induced by changes in host cells? The one most studied is the accumulation of substances toxic to parasites, phytoalexins, to concentrations sufficient to limit their growth as seen in resistant reactions. In uninfected tissues phytoalexins either are undetectable or present in concentrations insufficient to decrease significantly growth of the parasite. In resistance responses, they may accumulate as products of new biosynthetic pathways, of activated existing pathways which operate at much lower levels in normal cells, or because of decreased breakdown of phytoalexins in such pathways. Such evidence as there is indicates that phytoalexins are usually the products of new pathways. In view of the emphasis of past and current research on phytoalexins as the final cause of resistance it is surprising that there is so little quantitative evidence about rates and timing of their accumulation in resistant and susceptible reactions and whether these can explain differences in growth of a parasite. Much of the evidence is not much more than the demonstration that phytoalexins do accumulate more rapidly in resistant than in susceptible responses.

Phytoalexins act in resistance because they are toxic to parasites. If one or more do not accumulate sufficiently, then clearly something else must decrease the growth of the parasite. Lignification or closely related changes in cell walls has been studied in this context but only in a few diseases. No doubt there are also other mechanisms particularly in resistance to obligate parasites. The rest of the paper will be confined largely to phytoalexins as causes of resistance but almost all would apply to other mechanisms.

1.1 *Cell damage and phytoalexins*

In resistant responses phytoalexins accumulate in and around cells damaged and usually killed by the parasite. But almost from the start of research on phytoalexins it

has been known that phytoalexins may accumulate at similar rates following damage caused by other agents. Thus a bewildering variety of substances cause pisatin to accumulate in pea tissues. Substances may have these effects in concentrations that do not kill plants cells; UV radiation may act similarly. Therefore it seems that accumulation, probably because of new or greatly increased synthesis, follows damage to cells of which that caused by parasites is only one of the many forms, and thus I come to elicitors a word introduced some years ago for substances which elicit responses associated with resistance. "To elicit" means to "draw forth (what is latent; usu. fig.); draw out, evoke, (admission, answer *from* person)" (The Concise Oxford Dictionary, 1976). In the context of resistance it means not much more than "to cause" and "an elicitor" essentially means "a cause". Nevertheless there need be no harm and there can be some benefit from using elicitor in relation to resistance so long as the way in which it is being used makes clear that which is being elicited. Usually this is or is assumed to be the accumulation of phytoalexins.

It is comparatively easy to obtain from fungi, pathogens or otherwise, substances which in low concentration elicit the accumulation of phytoalexins when applied to plants. Most of them are polymers, carbohydrates or glycoproteins, which also are toxic to plant cells. Potent among these elicitors are branched β-glucans similar to those known to be major structural components of fungal cell walls (Albersheim and Valent 1978). Such elicitors can be extracted by drastic chemical treatment. Similar substances may also occur in culture fluids probably after autolysis of the cell walls. Much lower molecular weight products of degradation of such glucans also are active elicitors. Chitosan, a polymer of β-1,4 linked glucosamine residues is also an active elicitor (Hadwiger and Loschke 1981). An interesting recent finding is that β-1,3 glucanases from soybean tissues rapidly release from cell walls of *Phytophthora megasperma* f. sp. *glycinea* a glucomannan which elicits accumulation of glyceollin in soybean tissues (Keen and Yoshikawa 1983).

Interesting as are these glucans both as elicitors of the accumulation of phytoalexins and for their toxicity to plant cells at low concentrations, they are unlikely to be important in race-specific resistance simply because they are not specific in their activity. Such elicitors from races of a pathogen have similar effects on differential cultivars of a host plant and may even be active on non-host plants. Also, glucan elicitors have been isolated from yeasts.

Certain glycoproteins from fungi are also active elicitors. Evidence for their specificity has been obtained for the following: soybean and *P. megasperma* f. sp. *glycinea* (Keen and Legrand 1980) or *Pseudomonas glycinea* (Bruegger and Keen 1979); *Phaseolus vulgaris* and *Colletotrichum lindemuthianum* (Anderson 1980); *Pisum sativum* and *Fusarium solani* (Daniels and Hadwiger 1976). These glycoproteins have not been characterized and their role in the diseases remains to be established. Another glycoprotein has been isolated and characterized from *Rhizopus stolonifer* as an *endo*-polygalacturonase which is an active elicitor of the synthesis of casbene in castor bean (*Ricinus communis*) (Lee and West 1981). But *R. stolonifer* is no more than a weak pathogen, usually of storage tissue, so this interesting work is not directly relevant to race-specific resistance. Similar results have been obtained for an *endo*-pectate *trans* eliminase from *Erwinia carotovora* (Davis *et al* 1982). Such enzymes rapidly kill plant cells so it is possible that it is death of cells that leads to the synthesis and accumulation of phytoalexins in ways to be referred to later; such activity would be very largely non-specific. These enzymes rapidly degrade pectic polysaccharides in cell walls

to lower molecular compounds which are known to be active elicitors (Bruce and West 1982; Northnagel *et al* 1983). Other substances may also be released from cell walls by these enzymes. But again their activity is likely to be non-specific certainly in the context of race X cultivar systems.

The most promising research so far reported is for races of *Fulvia fulva* (*Cladosporium fulvum*) and cultivars of tomato (De Wit and Spikman 1982). Cell-free extracts from leaves infected with virulent races contained substances which when applied to appropriate cultivars caused necrosis and chlorosis in patterns similar to those caused by the races themselves. The specifically active substances are produced in leaves irrespective of genotype so long as they are susceptible (De Wit *et al* 1984). It is to be hoped that in spite of formidable technical difficulties more will soon be reported about the activity and nature of these substances because the lack of progress in isolating elicitors that act specifically has been frustrating particularly in light of the efforts that have been made and the expertise of those who have made them. On present evidence the best candidates for elicitors seem to be glycoproteins with specificity based on the carbohydrate moieties the structure of which would be determined by glycosyl transferases coded for by genes for avirulence in races of a pathogen (Albersheim and Anderson-Prouty 1975). Other possibilities would be of carbohydrates with specific activities which are released from the pathogen by host enzymes or from host cell walls by enzymes of the pathogen. Little as is known about the products (elicitors) from races that determine their specificity still less, indeed almost nothing, is known about the complementary products in cultivars. It has been suggested that elicitors act directly on host DNA and in so doing depress transcription of one or more of the genes involved in synthesis of phytoalexins (Hadwiger and Schwochau 1969). But comparison with other systems suggests that plasma membranes are more likely to be sites of the products that react with elicitors and there is some evidence that they are (Yoshikawa 1983). If so there is the problem as to how a reaction in the plasma membrane leads to the synthesis of phytoalexins. This would require the formation of a secondary messenger that conveys information to the host cell nucleus followed by transcription of the genes controlling synthesis of phytoalexins. This second messenger may be constitutive elicitor which is released from plant cells damaged by freezing or in other ways and which causes accumulation of phytoalexins when applied to plant tissues (Hargreaves and Bailey 1978). One asks whether elicitor should be used for such substances if the elicitor also is used for a primary product of a pathogen that causes them to be released; it could be confusing to use the same term for substances of different origin and function unless it were suitably qualified to emphasize these differences. Endogenous "elicitors" may also be released from plant tissues by chemical or enzyme treatments that yield low molecular weight pectic polysaccharides some of which can cause the accumulation of phytoalexins. What now needs to be investigated is the connexion, if any, between the primary response, probably in plasma membranes, and the release or activation of enzymes which produce low molecular weight products as secondary messengers. Whatever their origin and nature the secondary messengers almost certainly must be able to move from cell to cell, at least locally, because the available evidence suggests that phytoalexins are synthesized in a small number of cells around the cell in which the primary response occurs. They then are assumed to move back into the infected cell where they accumulate to prevent growth of the pathogen. Almost always this cell is dead and it is usually assumed that death is a primary response to the pathogen. But because certain phytoalexins are known to be quite toxic to plant cells

there is the possibility that it is the accumulation of phytoalexins that kills the cell containing the pathogen. There would then be the problem of why they do not kill the cells in which they are synthesized but which usually remain alive around the cell containing the pathogen. If in some diseases they did so and if these cells also released a secondary messenger that induced synthesis of phytoalexins in adjacent living cells then a lesion could develop containing many more cells than in a typical hypersensitive response and similar to certain leaf spots in which tissue occupied by the pathogen is smaller than the lesion itself. Necrosis in such diseases is usually attributed to toxins produced by the pathogen. Then one must ask why the pathogen does not colonize the dead cells and continue to kill more and thus cause a spreading rather than a restricted lesion. Is it possible that the agent that kills host-cells is the phytoalexin that confines the growth of the pathogen. If so, there remains the problem of the restriction in size.

A last point relates to the abiotic agents that non-specifically cause accumulation of phytoalexins. If the sequence of events leading to this starts in plasma membranes how does the damage caused compare with that caused specifically by products of *P* genes which require complementary products of *R* genes to be effective, and are the secondary messenger and subsequent events similar?

So much for the model upon which most research on race-specific resistance has been based. There is another model which has attracted much less research but which may attract more in the future. Thus in potatoes and *Phytophthora infestans*, certain water soluble, low molecular weight glucans from the pathogen suppress necrosis and accumulation of phytoalexins otherwise caused by high molecular weight substances from the pathogen which act non-specifically (Doke *et al* 1980). The suppressors were specific in that they suppressed resistant responses only in cultivars that were susceptible to the race from which they were obtained. There is the difficulty that non-specific elicitors from the pathogen are many times more active than are the specific suppressors but if this can be resolved for conditions *in vivo* then there will be good evidence that it is the suppressors that are the determinants of specificity, at least in this disease. If so would the model cope with the fact that genes for resistance to *P. infestans* are dominant and so probably are genes for low virulence? One explanation is that specificity depends on reactions between mutable suppressors from races of the pathogen which prevent the binding of a common, non-specific elicitor to mutable receptors in the host which would be the primary reaction of a hypersensitive response leading later to accumulation of phytoalexins. In the absence of this response the pathogen would grow in the host plant. The credibility of this explanation depends on the concept of a non-specific elicitor and conversion of a progenitor to a pathogen by production of a species-specific suppressor which establishes a "basic compatibility" between plant and micro-organism which will be referred to later.

1.2 *Host selective toxins*

There are now about ten diseases in which such toxins and their receptors in plants are considered to be the determinants of specificity in the following manner. A virulent pathogen of susceptible plant *X* produces a toxin which damages plant *X* at concentrations much lower than those at which it damages other plants which are resistant to the pathogen. Production of the toxin and of virulence may be controlled by one gene as is susceptibility to the toxin and to the pathogen; both genes are usually dominant. Therefore it is susceptibility and not resistance that is determined by a

reaction between the two gene products; otherwise the plant is resistant. In considering this explanation one should consider the behaviour of the pathogen in resistant and susceptible responses. The resistant response has all the features of hypersensitivity—death of one cell or of a few cells to which the pathogen is confined. What kills the cell? It cannot be the host-selective toxin. And if the pathogen by some other mechanism kills one cell why does it not proceed to kill many more in the same way? Unless, of course, the killing of the cell leads to other changes that prevent growth of the pathogen as described in §2.1. In susceptible reactions in certain diseases the pathogen may grow for some time and not kill cells as in other diseases in which host selective toxins have not been implicated. Later many host cells are killed, presumably by the toxin which kills at low concentrations only cells with a receptor coded for by the gene for susceptibility. But at this stage the selectivity is redundant. The toxin could as well be non-specific as it is in many other diseases. It has also been suggested that the host-selective toxin first acts in susceptible plants by suppressing the hypersensitive response thus allowing the pathogen to grow and produce more toxin. In which case one must ask about the levels of toxin sufficient to suppress the hypersensitive response but insufficient to kill susceptible cells. Another puzzling feature of Victoria blight of oat and the host selective toxin victorin of *Helminthosporium victoriae* is that the gene-controlling susceptibility to the toxin is probably at the same locus as the gene controlling resistance to races of *Puccinia coronata* which have the matching gene for low virulence. How does the product of this gene compare with the toxin controlled by the gene for virulence in *H. victoriae* if they have a common receptor in oat plants? It seems to me that the explanation of resistance and susceptibility in diseases caused by pathogens that do produce host-selective toxins may not be so simple as commonly has been assumed.

2. Race non-specific resistance

In the above sections diseases in which resistance and susceptibility are controlled by pairs of matching genes have been considered. Despite this simplest of relationships we know little about how reactions between different gene products in pairs lead to a common mechanism of resistance and on how the same mechanism is induced by abiotic agents. The type of resistance now to be considered has been far less studied. It has been called race non-specific, minor gene, polygene, adult plant, durable, horizontal to name most of the terms. For present purposes the most useful refers to the lack of specificity between races and cultivars, race non-specific, which usually depends on more than one gene, usually many and undetermined. How do these genes control the mechanism of resistance in the light of race specific resistance, the only other system about which more than a little is known, and in which resistance is controlled by reactions between specific products of genes in pairs, $P_1 \times R_1$ or $P_2 \times R_2$ and so on, which do something that limits disease. This something may be the same for different gene pairs such as accumulation of the same phytoalexin ($P_1 \times R_1$ and $P_2 \times R_2 \rightarrow X$) or it may be different as when cultivars with different genes for resistance react differently to races of *Erysiphe graminis* f. sp. *hordei* ($P_1 \times R_1 = X_1 P_2 \times R_2 = X_2$ and so on). Does polygenic resistance compose a series of matching genes for low virulence (Pm) and for resistance (Rm). If so does $Pm_1 \times Rm_1 \rightarrow Xm$ and $Pm_2 \times Rm_2 \rightarrow Xm$ and so on such that ΣXm is the mechanism of resistance and is less than X as almost always it is in

polygenic compared with monogenic resistance. Or does $Pm_1 \times Rm_1 \rightarrow Xm_1$ and $Pm_2 \times Rm_2 \rightarrow Ym_2$ and so on where Xm_1 and Ym_2 are different responses in which case what does $\Sigma Xm_1 + Ym_2 \ldots$ amount to, or do $Pm_1 \times Rm_1$ and $Pm_2 \times Rm_2$ cause effects quite different from those caused by $P_1 \times R_2$, $P_2 \times R_2$ in monogene systems? A related point is the one made some years ago along the following lines. In the absence of $P \times R$ let resistance be X_0 which is susceptibility. For $P \times R$ let resistance be X which is high. Reaction $P_1 \times R_1$ gives Xm_1 which is much lower than X but significantly higher than X_0. If this happens for a succession of p genes then polygenic resistance may be ΣXm dependent on many relic R genes now matched by complementary p genes. This could also explain in part the persistence of this type of resistance in that such genes for low virulence would be unlikely to change to genes for higher virulence as they do so often in race-specific resistance. In biochemical terms it would be possible to speculate about the effects of the different genes in polyenic resistance in terms of gene dosage, changes in composition of gene products, resulting in different affinities, rates of reactions and so on but at present there is very little data upon which to base such speculations.

Finally under this heading may be considered the resistance of "land races", the cultivars of less highly developed farming. They respond to pathogens much as do cultivars with race non-specific polygenic resistance which is probably land race resistance not lost in plant breeding programmes as other properties have been changed. But this resistance is sometimes lost. How does this happen if the effect of each of the many genes is small, even if each confers no more than a slight disadvantage in the absence of the pathogen. If land race resistance is polygenic resistance which depends on many genes each contributing to resistance then how does it relate to the resistance of the wild plant progenitors of the land races to common pathogens bearing in mind that wild plants carry many genes which confer high race specific resistance when transferred singly to crop plants but to which pathogens often and quickly become virulent. Also, it is now known for a few pathogens that such genes may function in wild plants much as they do in crop plants. The implications of findings such as these for a better understanding of race non-specific resistance should stimulate much more research on the behaviour of plant pathogens in populations of wild plants.

3. Non-host resistance

Although a minority of pathogens cause disease in many species of plants which are not closely related the great majority are highly specialized parasites of only relatively few of the vast range of plant species that potentially are available. Almost always a plant species is resistant to all micro-organisms except the few that are its pathogens. This has come to be called non-host resistance. It is of course by far the predominant form of resistance in wild and cultivated plants and it is also the type least studied because there seems rarely to have been the inclination and still less the money to investigate why a micro-organism is not a pathogen. Also, for non-host resistance there is the overriding difficulty that the genetics of crosses between a host and non-host plant almost always cannot be investigated. Nevertheless there is no good reason why this resistance should not be studied in other ways.

An interesting point about non-host resistance is that it was the basis of some of the earliest work which led to the concept of phytoalexins. And it is perhaps surprising that the earliest responses and the later accumulation of phytoalexins are, at least in some

non-host responses, similar to those in race × cultivar resistance where the product of race gene P_1 × product of cultivar gene $R_1 \rightarrow X_1$ the mechanism of resistance. In non-host resistance in which X_1 may be similar what replaces $P_1 \times R_1$? Are there multitudes of genes corresponding to P_1 in each micro-organism to match multitudes of complementary genes corresponding to R_1 in all the non-host plants with all the complications and ramifications which this would imply (Wood 1976). This seems improbable but how much evidence is there to refute it? Other hypotheses are based on an assumption that all plants have mechanisms for recognizing and reacting against non-self including microorganisms. Therefore, to become a pathogen of a particular plant a microorganism either must not induce this non-specific response or must later nullify its effects and thus allow a "basic compatibility" to be established; gene-for-gene systems are a later development (Bushnell and Rowell 1981; Ellingboe 1976; Heath 1982). I have raised elsewhere the difficulties of reconciling the postulated and general response of plants with the host of specific "basic compatibilites" *i.e.* all the diseases of plants, other than by schemes which are as complex as hypotheses based on gene-for-gene responses (Wood 1984). In the same paper I also considered briefly the following problems about non-host resistance: how does a saprophyte which is not a parasite of any plant differ from a highly specialized parasite which is not a parasite of all but a few plants; how do saprophytic members of a genus e.g. *Penicillium* or even of a species e.g. *Fusarium oxysporum*, differ from members that are specialized parasites; what happens when an isolate of a highly specialized parasite loses its pathogenicity so that it becomes essentially a saprophyte; stating that a microorganism becomes "compatible" with a plant, in other words a parasite, means that before this happened it was a non-parasitic symbiont (and therefore compatible in the better sense of the word) or a saprophyte. It is, perhaps not difficult to imagine the transition from symbiosis but much more difficult for the very different life styles of saprophytes and parasites. One hopes that these and other problems of non-host resistance will attract a much larger proportion of research on resistance than they have in the past but I doubt that they will.

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Spore germination in the higher Basidiomycetes

NILS FRIES

Institute of Physiological Botany, University of Uppsala, Box 540, S-751 21 Uppsala, Sweden

Abstract. This survey of the spore germination requirements in the Hymenomycetes and the Gasteromycetes shows that saprophytes as xylophiles, which decompose wood and forest litter, and coprophiles, which live on dung, usually germinate easily even on simple nutrient media. Species forming ectomycorrhiza with trees or living as parasites require as a rule more particular conditions for germination. In the mycorrhiza-formers, chiefly agarics and boleti, germination can often be induced by exudates from tree roots or certain yeasts, in species of *Leccinum* by exudate from self mycelium. The heartrot fungi, chiefly those species of Aphyllophorales which are parasites on trees, germinate preferably when exposed to an increased CO_2 content in the air or to exudates of certain micro-organisms. In many praticolous fungi, which are supposed to parasitize roots of grasses and herbs, germination is stimulated by various yeasts. The possibility of interpreting these particular germination conditions as adaptations to a parasitic or symbiotic life is discussed.

Keywords. Xylophilous fungi; coprophilous fungi; praticolous fungi; ectomycorrhiza-formers; root exudates; activated charcoal.

1. Introduction

In the higher Basidiomycetes, *i.e.*, the Hymenomycetes and the Gasteromycetes, dispersal is essentially based on the sexually produced basidiospores. Basidiocarp formation and basidiospore germination represent two critical events in the complete life cycle of these fungi. Both processes are accomplished by a sometimes very delicate interplay between internal and external factors, which is often difficult to analyse and understand. In this article I shall only deal with spore germination in the higher Basidiomycetes and try to present a summary of our present knowledge in this field. Ecological aspects will be particularly considered. Complete coverage of the relevant literature is out of the question in a survey of this rather limited extent and the selection must inevitably be somewhat arbitrary, although focussing on groups where induction of spore germination is still problematic.

The germination of spores has always been and is still studied principally with the aid of a light-microscope. A spore is usually said to be germinating when a germ hypha or a germ vesicle can be observed. For practical reasons many researchers state, arbitrarily, that a spore, to be scored as germinated, must have produced a germ hypha of a certain length, for instance as long as it is broad or of a length equal to half the diameter of the spore.

As regards germination it should be pointed out that viability is not the same as germinability. A spore may be viable, *i.e.*, living, without being able to germinate, because germination in this case only occurs under very special environmental conditions, which are not present. On the other hand, germination takes place of course only with viable spores. Viability and germinability always gradually disappear with time. Therefore it is often desirable to find out how many spores in a stored

collection are really alive, before a germination experiment is started. This is usually done by testing the enzymatic activity in the spore using suitable fluorescent stains (*e.g.* Stack *et al* 1975; Yu and Trione 1983; Sutherland and Cohen 1983).

In most of the investigations reported in this review the spores were germinating on nutrient agar plates, sometimes in drops of a liquid medium, under sterile (axenic) conditions.

In this survey scientific names of fungi are given in the form they were used by the quoted author. In some cases the name most current at present is added within brackets.

2. The pioneers in the study of homobasidiospore germination

Brefeld, the great pioneer and master in the art of growing fungi under controlled conditions, summarized in one of his last publications the main results of his numerous laboratory experiments on spore germination in different ecological categories of Basidiomycetes (Brefeld 1908). He stated that germination of basidiospores from fungi growing on dead wood and litter, which they decompose, generally takes place without difficulty even on very simple substrates, as water and water agar. He had also found that fungi living on animal droppings and manure germinate easily. These two categories, the xylophilous and the coprophilous fungi, are typical saprophytes, which utilize dead, organic substrates as sources of carbon and energy.

In contrast to these fungi, the soil-inhabiting Basidiomycetes, nowadays recognized chiefly as ectomycorrhiza formers with trees, had always proved to be very refractory in Brefeld's germination experiments. Evidently he based his conclusion on a large number of unsuccessful experiments with these fungi. Equally negative were the results of earlier and contemporary mycologists. Rarely were any experiments with soil-inhabiting fungi explicitly mentioned, but the negative outcome of such experiments may be inferred from the fact that the publications in this field generally deal with xylophilous and coprophilous Basidiomycetes, whereas the well-known mushrooms and toadstools are passed over in silence. Eidam (1875) is an exception in this respect, since he described his fruitless attempts to germinate spores of *Amanita*, *Lactarius*, *Russula*, and *Boletus*.

New attempts were made during the first three decades of the present century to solve the problem of spore germination in the soil-inhabiting or rather the ectomycorrhiza-forming Basidiomycetes. Again, almost all of these attempts were unsuccessful (Duggar 1901; Ferguson 1902; Cool 1912; Levine 1913; Kniep 1913; Romell 1921; Melin 1922; Hammarlund 1923; Vandendries 1933). Only Fuchs (1911) reported positive results, which seem to be the first reliable ones reported in this field of research. Although most of his experiments failed he managed to get germination of spores from the typical ectomycorrhizal fungus *Lactarius delicious* and also of spores from *Hydnum imbricatum*, which in all probability forms ectomycorrhiza with conifers. However, his results with *L. deliciosus* have been called in question by Oort (1974).

As will be reported in the following pages, efforts made by several researchers in this difficult field during the last half-century have led to some, yet still limited, success.

As regards the two other ecological groups recognized by Brefeld, the xylophiles and the coprophiles, almost every year during the present century has added new examples of species that conform to his rule of easy germinability. The only noticeable

exceptions have been found among wood-destroyers, which live as parasites on trees.

Finally, it has proved motivated to add a fourth ecological category, the praticolous fungi (Parker-Rhodes 1951), to the three just mentioned. The praticolous species were counted among the soil-inhabiting fungi by Brefeld, and as such they had demonstrated their unwillingness to germinate in the laboratory. These fungi occur on open fields and are not ectomycorrhiza formers. Their mode of nutrition is still largely unrevealed and so are also their requirements for germination on artificial media in the laboratory.

3. Spore germination in different ecological groups

3.1 *Xylophilous fungi*

Spore germination of a considerable number of xylophilous Basidiomycetes had been observed and studied even early in the 19th century. There is not space here to enumerate all those workers who about a hundred years ago contributed to an increased insight into the life cycle of wood-decomposing fungi. Only a few outstanding names are H Hoffmann, E Eidam, Ph. van Tieghem, and O Brefeld. More detailed information on this period can be obtained elsewhere (see Bavendamm 1936; Fries 1943 for more details).

After the turn of the century the interest in these generally easily cultivated fungi increased, partly because of their economic importance as wood-destroyers. Later, when Bensaude (1918) and Kniep (1920) discovered the sexuality in Basidiomycetes, an interest in the genetics of these fungi arose. Monosporous cultures were a necessary prerequisite for such studies and therefore the xylophilous—together with the coprophilous—fungi became objects of choice because of their readiness to germinate. Furthermore, since the 1940's taxonomists have increasingly made use of crossing tests between monosporous mycelia to elucidate taxonomic relationships. Most of these studies were made with species belonging to Aphyllophorales, the dominating group among the xylophilous fungi. As an example may be mentioned Boidin's (1958) comprehensive, biotaxonomic investigation of 147 species and subspecies of Hydnaceae and Corticiacae. Spore germination occurred in 125 species and monokaryons could be isolated in 87. It is perhaps significant that in the Gasteromycetes, where the spores are usually difficult to germinate, the rather few easily germinating species are all found in the xylophilous and coprophilous families Nidulariaceae and Sphaerobolaceae (Hoffmann 1859; Hesse 1876; Eidam 1877; Brodie 1975; and others).

The number of xylophilous species which have been brought to germination *in vitro* is now immense. It may suffice to refer to some relevant overviews (Whitehouse 1949; Kneebone 1950; Merrill 1970).

It must be remembered that xylophilous fungi not only attack cellulose and/or lignin in such materials as trees, timber, and wooden houses, but also, although less conspicuously, the litter on the forest soils. Spore germination in these litter-decomposers, many of which are small agarics, also seems to be rather unproblematic.

Some of the rather few cases where germination *in vitro* has failed have led to efforts to elucidate the reason. In *Merulius* (*Serpula*) *lacrymans* some authors reported successful results (*e.g.*, Hartig (1885)), whereas others failed. The reason for the failure was evidently that the pH of the media used had been too high (Möller 1903; Falck 1912). Germination occurred on most media if a suitable amount of an organic or

inorganic acid was added, malic acid being recommended as particularly efficient (Findlay 1932; Harmsen 1960).

The only group of xylophilic Hymenomycetes which still presents serious difficulties for workers trying to induce *in vitro* spore germination, comprises some species of Polyporaceae, which live as parasites on trees. White (1920) found that *Polyporus applanatus*, which causes heartrot in various trees, never germinated at more than 1 % and sometimes not at all. Later workers found that up to 78 % germination could be obtained if the spores were situated close to growing mycelia of *Ceratocystis* sp. or colonies of yeasts and bacteria (Brown and Merrill 1973). The effect was caused by volatile substances, still unidentified, which were produced by the adjacent activator organisms.

In *Polyporus dryophilus* and related species the germination-inducing mechanism seems to be somewhat more complicated. Bailey (1941) reported that germination occurred only on a malt extract medium and only under the condition that a mycelium of the fungus had previously been grown on the medium. Spores also germinated in water drops to which the same medium, filtered and sterilized, had been added. However, Mog and Morton (1970) induced up to 92 % germination in this fungus by increasing the CO_2 content to 65 % of the air phase. Later, Morton and French (1974) showed that the effect of other microorganisms, especially *Ceratocystis fagacearum*, could not be due only to their CO_2 production but also to volatile organic emanations of unknown identity. In *Fomes rimosus*, another heartrot fungus, a maximum germination of 41 % could be obtained by keeping the spores in an atmosphere of 100 % CO_2. Tests with $^{14}CO_2$ showed that the carbon dioxide was fixed by the spores (Mog and Morton 1970). According to Hintikka (1970) the germination-promoting effect of an enhanced CO_2 content in the air is most pronounced in those wood-decomposing fungi which in nature inhabit living trees as parasites.

Among heartrot fungi, which have never germinated on artificial media, Merrill (1970) mentions *Fomes everhartii*, *Polyporus hispidus*, *P. lucidus*, and *P. tsugae*.

There are indications that microorganisms of various sorts influence the germination of xylophilous fungi not only under laboratory conditions but also in nature. Paine (1968) reported that spore germination in *Polyporus betulinus*, *Fomes pinicola*, and *F. subroseus* was higher on bluestained (*i.e.*, infected with blue-stain fungi) than on unstained branch stubs from all tested species of trees. Evidently some microorganisms inhabiting dead coniferous branch stub wood do not retard basidiospore germination but rather stimulate it. From this point of view it seems suggestive that bark extracts from diseased (infected) roots of spruce support better spore germinations in *Polyporus tomentosus* than do bark extracts from healthy roots (Whitney and Bohaychuk 1971). The positive effect of wood saprophytes on germination may not necessarily depend on production of stimulatory substances but on a removal of toxic compounds, as was shown by Carey and Savory (unpublished) in *Trichoderma viride* and blue stain fungi.

Similarly, water extracts from dead branches of aspen, the host species of *Fomes igniarius* var. *populinus*, stimulated spore germination of this parasite, whereas water extracts from living branches had no positive effect (Wall and Kuntz 1964). Whether the stimulatory substances in the dead branches had been produced by the tree or by microorganisms in the decaying wood could not be decided.

In certain truly saprophytic xylophiles germination requires special conditions as well. Boidin (1958) found that in a few species of Aphyllophorales germinating spores

were observed only together with colonies of bacteria belonging to the *Bacillus brevis* group.

Flammula (*Pholiota*) *alnicola* is one of the rather few xylophilous agarics which are difficult to germinate. It is a saprophyte on wood but lives sometimes also as a parasite on trees, which are infected through the root system. Denyer (1960) reported that germination on malt agar is slow and never exceeds 1 %. The germination percentage could be doubled if the spores were stored at $-7°C$ for 10 weeks to 13 months. Lower temperatures were inefficient. The closely related *F. conissans* reacted similarly, the effect of the cold treatment in this case being even more conspicuous. It would be interesting to test whether the spores also in these cases are susceptible to the influence of CO_2 and adjacent microorganism colonies.

As mentioned earlier, spore germination in the litter-decomposing xylophilous fungi generally takes place easily and without any particular pretentions on the medium. As examples may be mentioned two genera specialized on this mode of living: *Marasmius* (Lindeberg 1944) and *Mycena* (Fries 1949). Most tested species in both genera germinated even in distilled water and on water agar, the germination in some species starting within a few hours. Still, some authors have reported difficulties with species of *Mycena* (Quintanilha 1944; Quintanilha *et al* 1941), one reason possibly being that the concentration of ammonium ions in the nutrient medium used had been too high, since germination in some *Mycena* species is totally inhibited in this way (Fries 1949).

3.2 *Coprophilous fungi*

From a nutritional point of view the coprophilous fungi do not differ from the litter-decomposing xylophiles. Like those they are—as far as we know—capable of decomposing cellulose and/or lignin, although they avoid wood and prefer the less compact substrate offered by dung and manure, which is also richer in nitrogenous compounds.

Typical coprophilous species are those of *Coprinus*, *Panaeolus*, *Psilocybe* and *Bolbitius*. Since many of them form fruit-bodies in culture and grow rapidly they have been frequently utilized for genetic studies. Almost all of them germinate within a few hours or days and at a high percentage. This can be concluded from the fact that the geneticists working with these fungi very rarely have reported any difficulties with spore germination (Vandendries 1923; Brunswik 1924; Quintanilha 1944; Lange 1952; Kemp 1975).

There is one coprophilous fungus, however, whose spore germination has intrigued and has been studied by more mycologists than any other, namely the edible mushroom, *Agaricus bisporus*. The reason for this interest is its great practical importance as a commercially produced and appreciated vegetable and the difficulties early researchers met with in growing it from germinated spores. These difficulties are nowadays difficult to understand, since germination occurs easily on most common, slightly acidic, agar media if the spores have been incubated upon them for one to three weeks (Hoffmann 1860; Ferguson 1902; Falck and Falck 1924). Some failures might have been due to the occurrence of strains or morphologically slightly divergent species with poor germination capacity. Furthermore, the spores cast from one and the same fruit-body often differ considerably in germinability depending on the age of the fruit-body (Cayley 1936).

During the course of these early studies Ferguson (1902) observed that germination

started earlier if a piece of a growing mycelium was present close to the spores. This simple measure has now become a routine among workers in this field (*e.g.* Elliott and Wood 1978) who want to secure a satisfactory outcome of germinated spores. Further studies by Hutchinson and collaborators (Hutchinson 1971; McTeague *et al* 1959; Lösel 1964) revealed that the germination-stimulating substance exuded from the *Agaricus* mycelium was also produced by yeasts and other fungi. Being volatile it diffused through air to the spores in the vicinity. It could finally be identified as isovaleric acid. Its mode of action was skillfully elucidated by Rast and Stäuble (1970). Briefly, the isovaleric acid triggers germination of the spores by overcoming the self-inhibition caused by metabolically produced, internal carbon dioxide. By a carboxylation reaction this carbon dioxide is removed by being bound to β-methylcrotonyl-CoA, formed from isovaleric acid. As yet this is the only case where the biochemical mechanism behind the germination-inducing effect of a specific compound has become fully understood.

Volvariella volvacea is another coprophilous fungus which ought to be mentioned because of its particular spore germination requirements. This is, like *Agaricus bisporus*, an edible fungus, extensively grown in the tropics. Thriving well on decaying plant material, especially paddy straw, it has a less coprophilic character than *Agaricus* and may just as well be placed among the litter-decomposers. Chang and Chu (1969) found that spores placed directly on agar plates germinated relatively well, but that presoaking with water increased the percentage germination from *ca* 40 % - 85 %. The soaking effect was explained as either a breakage of permeability barriers or a washing off of inhibitory substances in the spores. The optimum germination temperature was as high as 40°C. This could be interpreted as a mild heat shock, stimulatory to spore germination in this species.

3.3 *Praticolous fungi*

The ecology of the praticolous fungi is insufficiently known. They are characterized by growing outside the forests, on meadows and lawns, and probably living on dead and dying roots of grasses and herbs. Their nutritional capacities have been studied chiefly on species close to the coprophilic group, e.g., *Agaricus* species. Typical representatives of the praticoles are the Lycoperdaceae (the puffballs) and many species of *Hygrophorus* among the agarics.

Although the borderline towards the other ecological categories, the xylophilous and coprophilous fungi, is diffuse, the praticoles distinguish themselves by being exceptionally adverse to germination *in vitro*.

The numerous and common species of Lycoperdaceae with their enormous basidiospore production resisted all attempts to induce spore germination for almost a century. Some claims of success proved to be false alarms and could not be repeated (Hoffmann 1859, 1860; Swartz 1928). Constantly negative results were obtained by, among others, Brefeld (1877), De Bary (1884), Ferguson (1902), Cool (1912), and Kaufmann (1934).

The first fruitful experiment was the result of a happy coincidence (Fries 1941). On a malt extract agar plate sown with spores of *Lycoperdon umbrinum* some contaminations appeared, probably originating together with the spores from the fruit-body. One of them was an unidentified yeast. Close to this developing yeast colony a few germinating *Lycoperdon* spores could be seen. Repeated experiments gave the same

result and also demonstrated that germination started after five days. Some other yeasts, especially species of *Torulopsis*, also proved capable of inducing germination. Thus, four more *Lycoperdon* species were germinated. Moreover, it was shown that malt extract agar plates on which *Torulopsis*, or some other red yeast, had grown permitted germination of *Lycoperdon* spores even after removal of the yeast from the surface and after a renewed autoclaving of the substrate. From experiments with synthetic nutrient media it could be concluded that the activator yeast not only produced a germination-inducing substance (or substances) but also eliminated a germination-inhibiting compound, which otherwise could be removed only by a thorough rinsing of the agar before autoclaving. Similar effects, but less striking, were produced by some filamentous fungi.

Some twenty years later Bulmer and Beneke started a more comprehensive investigation of the spore germination conditions in Gasteromycetes. Through their careful and elaborate experiments much new and valuable information was obtained (Bulmer and Beneke 1961, 1964; Bulmer 1964). With red yeast, mainly *Rhodotorula mucilaginosa*, germination was induced in about 50 species of Gasteromycetes, the majority being praticoles of the genera *Calvatia*, *Bovista*, and *Lycoperdon*. In all cases the percentage germination was extremely low, rarely above 0·1 %. Interestingly enough, germination was observed in *Calvatia gigantea* even without *Rhodotorula* if the spores were incubated in shake culture flasks with barley extract medium or *Calvatia* extract medium. After such treatments approximately 1 spore germinated out of 20 millions. In agar plate tests malt extract was the most efficient component, and with *Rhodotorula* up to 3 spores per million germinated in *Calvatia gigantea*. The spores proved capable of retaining their germinability for several years in the dried sporophores.

Later experiments by Wilson and Beneke (1966) corroborated some of these results, notably the importance of malt extract, and gave new information on the role of carbon and nitrogen sources in the germination process. Several species of *Rhodotorula* were tested for activity. Not only red strains, but also yellow ones, promoted germination in *Calvatia gigantea*. Generally, however, the species were the more active the redder they were.

When turning to the praticolous agarics very little can be reported of their modes of basidiospore germination. Among the dominating genera within this ecological category the following may be mentioned: *Clitocybe*, *Entoloma*, *Nolanea*, *Galera*, and *Hygrophorus* (Parker-Rhodes 1951). According to my own (unpublished) experiences, the *Hygrophorus* species differ widely from each other as to their ability to germinate *in vitro*. In *H.* (*Camarophyllus*) *niveus-virgineus* group as well as in *H.* (*Hygrocybe*) *psittacinus*, spores often germinate on synthetic nutrient agar without any activator organism. The germinability of the spores seems to differ considerably from one basidiocarp to another. In *Clitocybe* and *Hygrophorus* it is often hard to judge which species are really true praticoles; some may be facultative ectomycorrhiza formers.

3.4 *Ectomycorrhiza formers*

Most of Brefeld's soil-inhabiting fungi are nowadays recognized as ectomycorrhiza formers with forest trees. They represent the great majority of the species in certain genera of Agaricales (e.g., *Tricholoma*, *Amanita*, *Inocybe*, *Hebeloma* and *Cortinarius*), of Russulales (*Russula* and *Lactarius*), and almost all genera of Boletales (e.g., *Boletus*,

Suillus, *Leccinum*, *Paxillus*, and *Gomphidius*). Some representatives are found in the Gasteromycetes (e.g., *Scleroderma* and *Rhizopogon*) and the Aphyllophorales (e.g., *Thelephora*).

As mentioned earlier, Fuchs (1911) was the first to see germinating spores of a typical ectomycorrhiza-forming fungus, namely *Lactarius deliciosus*. The germination evidently occurred without any pretreatments of the spores or any particular supplements to the medium. Nobody seemed to have been able to repeat it, until Kneebone (1950) induced *Lactarius luteolus* to germinate after having stored the spores at $-6°C$ for 135 days.

Hammarlund (1923) spent several years in attempts to germinate *Boletus* (*Suillus*) *grevillei* by trying innumerable combinations of nutrient media and variations in other environmental conditions. Germinating spores were found in three experiments, but apparently without any demonstrable correlation to the prevailing conditions. The results could not be repeated.

As a consequence of the experiments with praticolous Gasteromycetes already described, the effect of yeast colonies on germination was tested by Fries (1941) also with mycorrhizal species, like *Suillus luteus*. These experiments met with success and their simple technique proved applicable to other mycorrhizal species within several genera of Agaricales and Boletaceae. Thus, thanks to the germination-inducing power of *Torulopsis sanguinea* (and later *Rhodotorula glutinis*), germinating spores and monosporous mycelia could be obtained from several species belonging to the genera *Amanita*, *Tricholoma*, *Clitopilus*, and *Suillus* (Fries 1943).

However, the germination induced in this way was usually characterized by being slow and sparse, the percentage seldom exceeding 1%. There were still many mycorrhizal fungi which did not respond at all to the influence of *Rhodotorula*. Since some circumstances indicated the presence of inhibitory factors in the nutrient agar media employed, steps were taken to identify and remove these inhibitors. First it was found in experiments with species of *Suillus* that germination is very sensitive to the content of ammonium ions in the substrate. A radical reduction of the ammonium concentration considerably improved both rate and percentage of germination in *Suillus* (Fries 1976) and, as was later found, also in other genera.

Later it was discovered that germination in various ectomycorrhizal fungi was prevented by an inhibitor in agar which is formed from agarose during the autoclaving of the agar medium. The inhibitor could be removed by activated charcoal (Fries 1978) and has been identified as a weak organic acid chiefly active on mycorrhiza-forming Hymenomycetes (Bjurman 1984). This inhibitor is not identical with the one earlier mentioned, which is not formed from agar by autoclaving and affects germination in *Lycoperdon* (Fries 1941).

By relieving the nutrient agar media from these inhibitors and using *Rhodotorula glutinis* as an inductor organism, germination could be induced in a further number of ectomycorrhizal species, e.g., *Laccaria laccata* (Fries 1977), *Lactarius helvus*, *Paxillus involutus*, *Leccinum scabrum* (Fries 1978), and *Cantharellus cibarius* (Fries 1979b), and also improved the rate of germination in many others. The practising of these principles proved to be particularly fruitful in the Boletaceae genera *Boletus*, *Leccinum*, *Tylopilus*, and *Suillus*, where 23 of 25 tested species could be brought to germination (Fries 1983 c) (table 1).

It is evident that there are great differences among the many genera of ectomycorrhizal fungi as regards their response to both inhibitory and stimulatory factors. Oort

Table 1. Effect of plant roots, red yeasts (*Rhodotorula*) and self mycelium on the germination of basidiospores of ectomycorrhizal fungi. (Revised from Fries 1981b).

	No. addition		Pine or birch seedling roots		*Rhodotorula glutinis*		Self mycelium	
Species	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Leccinum spp.[1]	−	−	−	−	−	(+)	−	+
Thelephora terrestris[7]	−	−	+	+	(+)	(+)	(+)	(+)
Paxillus involutus	−	−	−	+	−	+	−	+
Cantharellus cibarius	−	−	−	−	−	+	−	(+)
Lactarius spp.[2]	−	−	−	−	−	+	−	−
Hebeloma spp.[3]	(+)	−	+	+	−	−	−	−
Suillus spp.[4]	(+)	(+)	·	·	+	+	−	(+)
Laccaria laccata	−	−	·	·	−	+	−	+
Tricholoma spp.[5]	+	+	·	·	·	·	·	·
Russula spp.[6]	−	−	−	−	−	−	−	−

+: germination; (+): sparse or slow germination; −: no germination; ·: not tested. *a*: agar medium not treated with charcoal. *b*: agar medium treated with charcoal. [1]: *Leccinum scabrum*, *L. holopus*, *L. aurantiacum*, *L. versipelle*, *L. variicolor*, *L. vulpinum*. [2]: *Lactarius helvus*, *L. torminosus*, *L. deterrimus*. [3]: *Hebeloma mesphaeum*, *H. crustuliniforme*. [4]: *Suillus luteus*, *S. granulatus*, *S. variegatus*. [5]: *Tricholoma equestre*, *T. imbricatum*, *T. pessundatum*, *T. terreum*. [6]: *Russula emetica*, *R. decolorans*. [7]: Birraux and Fries (1981).

(1974) found that some species of *Lactarius* germinated when exposed to volatile exudations from *Ceratocystis fagacearum*, a fungus which already has been mentioned as an inducer of germination in two xylophilous *Polyporus* species. Other *Lactarius* species responded positively to *Rhodotorula* and required charcoal-treated agar for a substrate (Fries 1981 b).

At the end of the 1950's Melin called attention to the influence of tree root exudates on ectomycorrhiza-forming fungi. These exudates promoted the mycelial growth rate in several species (the "M factor"), but also sometimes affected spore germination. He reported observations of germinating spores from species of *Suillus*, *Amanita*, *Lactarius* and *Paxillus*, when exposed to exudates from pine and tomato roots. When he used the same technique with spores of *Cortinarius* and *Russula* species his positive results were most impressive. Some *Russula* species responded to both pine and tomato roots, others only to pine roots (Melin 1959, 1962).

Many attempts have been made to repeat Melin's experiments, but always without success as regards the latter genera. However, there are other, later observations which also point to a germination-inducing effect of tree roots. The only existing picture of germinating *Russula* spores was taken from a flask culture of a birch plant, where the spores were situated close to the root surface (Heinemann and Gaie 1979).

In fact, germination-inducing effects of roots have been observed or presumed by several authors. Germination in *Hebeloma* spp. was strongly stimulated by pine roots (Fries and Birraux 1980) and germination in *Thelephora terrestris* by pine and birch roots (Birraux and Fries 1981). These experiments were performed on agar plates. Roots of herbs were inactive (table 1).

By adding spore suspensions to axenically grown tree seedlings ectomycorrhizae

were formed, which demonstrated that the spores—from species of *Scleroderma*, *Thelephora*, *Rhizopogon*, *Laccaria*, and *Pisolithus*—actually had germinated and formed mycelia, although the germination process could not be observed (Thapar *et al* 1967; Marx and Ross 1970; Theodorou and Bowen 1973; Stack *et al* 1975; Marx 1976).

The experiments made by Orlos and Twarowska (1965) are more difficult to interpret. They found that if spores of *Boletus edulis* and *Suillus luteus* were placed on glass slides and buried in forest soil, germination often took place after some time. For unexplained reasons it occurred only in certain experiments, however. These germinations could have been due to volatile or diffusible exudates from roots of the forest trees or from soil microorganisms.

The most striking example of an exceedingly specialized, but also very efficient, system for germination induction was found in the Boletaceae genus *Leccinum* (Fries 1979a, 1981a, 1973b). Like many other ectomycorrhizal fungi the *Leccinum* species can be brought to germination, although as usual a very poor one, under the influence of *Rhodotorula glutinis*. In contrast, a *Leccinum* mycelium growing among the *Leccinum* spores on an inhibitor-free nutrient agar plate induces germination of the spores within a few days at a percentage nearly always above 10% and often above 50%. However, a prerequisite is that the spores belong to the same species group as that of the mycelium: either to the *L. aurantiacum* (6 species) or to the *L. scabrum* (2 species) group. Crosswise combinations always give negative results.

Further investigations in this peculiar system have demonstrated that the germination induction is due to a non-volatile, diffusible pheromone produced by the mycelium and recognised by the closely related spores, which respond by developing a germination vesicle (Fries 1981a). The chemical constitution of this pheromone has not yet been identified.

In a search for analogous modes of germination induction in other genera than *Leccinum* I have studied the possible effect of self mycelium on spore germination in various mycorrhizal fungi. In some cases, *e.g.* in *Laccaria* spp., a positive influence on germination was observed, although unspecific and relatively weak, more like that of *Rhodotorula* (Fries 1983a).

Less spectacular but apparently without any equivalent among other mycorrhiza formers is the effect of amino acids on the spore germination in *Suillus*. In *S. luteus*, *S. granulatus*, and *S. variegatus* germination is strongly stimulated by casein hydrolysate and by particular mixtures of certain amino acids, glutamic acid being an especially active component (Fries 1976). How the amino acids affect germination is still an open question.

Only few ectomycorrhiza-forming hymenomycetes have been found which are capable of germinating on synthetic nutrient media without any activator organisms or without any special organic supplements. To these fungi belong some *Tricholoma* (Fries 1943) and *Hebeloma* species, in the latter case chiefly members of the section Denudata, subsection C (Bruchet 1973). After long incubation "spontaneous" germinations sometimes occur also in species of the genera *Suillus* and *Thelephora*.

Finally it should be mentioned that according to Voglino (1895) spores of *Inocybe*, *Russula* and *Lactarius* species eaten and digested by slugs germinate after passage through the digestive tract. The validity of his results has been called in question, however, and the experiments have never been successfully repeated (Ferguson 1902; Buller 1909).

4. Comments on some of the factors controlling spore germination and associated phenomena

4.1 *Duration of germinability*

The duration of germinability varies from one species to another and sometimes also within the species. A few examples may suffice to illustrate this.

Preservation at a sub-zero temperature is known to prolong the viability of the spores, at least in many species. Spores of *Lycoperdon pusillum* germinated even after 4 yr if stored at $-18°C$ (Bulmer and Beneke 1964). In this species the percentage germination slowly increased during storage up to an age of at least two and half years irrespective of the temperature. This occurred at all three storage temperatures tested: 26°C, 10°C, and $-18°C$. In *L. curtisii*, on the other hand, almost no spores germinated after two and a half yr, not even those which had been kept at $-18°C$.

In contrast, the spores of all tested *Laccaria* species lose their germination capacity within only three months, even if preserved at $-18°C$ (Fries 1983a). Species of *Suillus* remain germinable for about half a year at low temperature storage (Fries 1943) and species of *Leccinum* up to ten months (Fries 1978). In all these cases the germinability decreases faster if the spore prints are kept at a higher temperature, *i.e.*, at 4°C or 25°C.

In *Fomes igniarius* var. *populinus* studied by Good and Spanis (1958), spores from different basidiocarps differed considerably as to durability of germination power. Collections from some basidiocarps lost their germinability within 10 days, others after 40 days, whereas the spores from one of the basidiocarps (out of seven tested) still remained germinable after 80 days.

In a very comprehensive and careful study, Whitney (1966) demonstrated that spores of *Polyporus tomentosus*, when kept at $-18°C$, generally ceased to germinate after six to eight months, but before that they exhibited a percentage germination after three months which was higher than the original one. These results were based on average values from 332 tested spore collections. The variability in the collections could be extraordinary: in some of them no spores germinated, in others germinability was seemingly lost but returned after some months, etc. Still, Whitney's results suggest the existence of an after-ripening process in *P. tomentosus*, analogous to that noticed by Bulmer and Beneke (1964) in *Lycoperdon pusillum*.

However, real and total dormancy, which requires after-ripening or intervention of external factors to be overcome, has never been asserted in Basidiomycetes. It may be that *Flammula alnicola* and *F. conissans* represent exceptions to this rule, since they were found to germinate only after treatment at $-7°C$ (Denyer 1960).

It should be kept in mind that spores during storage may acquire an increased sensitivity to the conditions under which their germinability is tested. Watling (1963) demonstrated that herbarium collections of spores from some species of Bolbitiaceae only germinated if they had been incubated for several hours in a water-saturated atmosphere before they were plated out on nutrient agar.

4.2 *Intra-species and intra-basidiocarp differences*

As mentioned previously, spore collections from different basidiocarps within the same species often differ markedly in germination frequency and environmental requirements. Therefore, the characterization of a species from the point of view of

germination behaviour should not be based on experiments with a single spore collection only, but on several collections from different basidiocarps. As examples of this intraspecific diversity, references may be made to the studies by White (1920) in *Polyporus applanatus*, by Bulmer and Beneke (1961) in *Calvatia gigantea*, Bulmer and Beneke (1964) in species of *Lycoperdon*, Fries (1943, 1976, and unpublished) in *Amanita rubescens*, *Boletus* (*Suillus*) *luteus*, and *Hygrophorus* species, and Whitney (1966) in *Polyporus tomentosus*.

The rate of germination may vary not only among different basidiocarps from the same species, but also among different parts of the hymenium in one and the same basidiocarp. Cayley (1936) observed that in *Psalliota* (*Agaricus*) spp. the germinability of spores depends on the age of the basidiocarp when the spores were cast. Whitney (1966) found the same in *Polyporus tomentosus*, where the best spore production was from 4-week-old basidiocarps and the best germination was in spores from 3-week-old basidiocarps. In *Lycoperdon pusillum*, on the other hand, Bulmer and Beneke (1964) could not find any significant differences in germinability among spores from different areas (top, side, and bottom) of the basidiocarps studied.

4.3 *The role of some physical environmental factors*

In the higher Basidiomycetes the optimum temperature for spore germination as a rule seems to be about the same as the optimum temperature for mycelial growth (*e.g.*, Merrill 1970; McCracken 1982). An exception is the coprophilous *Volvariella volvacea*, which has an optimum germination temperature of 40°C (Chang and Chu 1969). The spores of the similarly coprophilous *Cyathus stercoreus* (Gasteromycetes) require two days incubation at 40°C for good germination (Brodie 1975).

The importance of a low storage temperature for the maintenance of germinability has already been discussed.

The influence of light on spore germination has been very little studied in these higher fungi. Apart from some old, occasional observations on the harmful consequences of direct sunlight, the only methodical investigation of light effects on spore germination seems to have been made by McCracken (1982) with *Pleurotus sapidus*. He showed that in this fungus germination is strongly inhibited by white and blue, but not by yellow and red light. Thus, wavelengths below about 500 nm inhibit germination.

4.4 *Interactions between spores and hyphae*

In the foregoing several cases have been mentioned where germination of spores has been induced by substances produced from mycelia, yeast colonies or bacteria in the vicinity of the spores. It ought to be added that chemically transmitted influences sometimes also go the other way: germinating or germinable spores produce substances which entice hyphae nearby to grow towards the spore, evidently by a positive chemotropic reaction. This mode of reaction was first observed by Morton and French (1970) in *Polyporus dryophilus* and by Bistis (1970) in *Clitocybe truncicola*. It was later found also in *Schizophyllum commune* (Voorhees and Peterson 1983). The change of growth direction of the attracted hypha (or hyphae) leads to a conjunction between the hyphal tip cell and the spore, probably ending up with plasmogamy. The phenomenon (with oidia as attractants) was further studied in many species of

Coprinus by Kemp (1975, 1977), who described the reaction as "homing". It also occurs among ectomycorrhizal Hymenomycetes, as *Leccinum* (Fries 1981a), *Laccaria* (Fries 1983a), and *Thelephora* (Birraux, unpublished). In the present context there is no reason to go into greater detail, but reference may be made to the interesting correlations found between homing reactions on one hand and taxonomy and genetics on the other (Kemp 1977; Fries 1983b). So far it seems that homing occurs only with living spores, and in *Leccinum* spp. only with spores which have already germinated.

Another case of interplay between spores and hyphae is the recently discovered reaction which is provisionally called "sporophagy" (Fries, unpublished). As yet it has only been observed and studied in the higher Basidiomycetes, but might well occur also in other groups of fungi. Sporophagy implies that hyphae of one species attack basidiospores of another species, twist around them, and to all appearances penetrate the spore wall. About 20 sporophagous species have as yet been found, most of them xylophilous. Dead spores are not attacked, nor hyphae, whether alive or dead. It appears that relatively large and coloured basidiospores are most attractive.

Group-size effects among densely sown spores are well-known in some Phycomycetes and Fungi imperfecti. They manifest themselves as mutual germination stimulations or inhibitions (for a review see Robinson 1973). In the higher Basidiomycetes such effects seem to be less common. However, according to Kühner (1938) spores of certain species of *Mycena* germinate rapidly only when they were sown out densely on the agar surface. More isolated spores may germinate but the germ hypha soon stops growing and dies. Boidin (1958) made similar observations with some xylophilous species of Aphyllophorales. This need of density for germination made the isolation of monosporous mycelial difficult. In *Calvatia gigantea* both very low and very high spore densities were unfavourable for germination, the optimum being between 1 and 5 million spores per agar plate (Bulmer and Beneke 1962).

5. Conclusions

The present, accumulated knowledge of spore germination in the Homobasidiomycetes gives a picture of striking diversity as far as germination conditions are concerned. This diversity is most pronounced among those fungi which in nature live as parasites on, or as symbionts with, higher plants, as is the case with the heartrot fungi and the ectomycorrhiza formers. Many of the praticolous mushrooms and puffballs, whose ecology is still little understood, have also proved to require very special conditions for germination and could therefore, per analogiam, be supposed to live as parasites on herb or grass roots.

Would it therefore be justified to interpret these special requirements for germination as adaptations to a parasitic or symbiotic mode of life? In several cases such interpretations have been suggested. The spores of *Flammula alnicola* and *F. conissans* attain their optimal germination if stored at $-7°C$ for 10 weeks up to one year, which fits in with their mission of spending a winter in the soil and then attacking a tree-root in the spring or the summer (Denyer 1960). In *Polyporus dryophilus* the spores require an increased CO_2 content in the ambient atmosphere, which can be found in their normal infection court: the decaying wood in branch stubs of living trees (Jensen 1969; Morton and French 1974). This seems to be the case with tree parasites in general (Hintikka 1970). In *Fomes igniarius* var. *populinus* the germination of the spores is

stimulated by substances present in the dead branches of the aspen tree, the host of this fungus (Wall and Kuntz 1964). Other varieties of *F. igniarius*, which are parasites on other trees, are inhibited in their germination by the same substances from aspen wood. Selectively stimulatory or antifungal substances may thus be a factor favouring or preventing the establishment of an infection via dead branches or stubs of a tree. When leaving the basidiocarp the spores of *Fomes applanatus* carry with them numerous infections, some of which are known to stimulate spore germination (Brown and Merrill 1973). It is also attractive to consider the cases of spore germination induced by root exudates, which occurs in some ectomycorrhizal fungi, as an adaptation to symbiotic life. More examples can be found in the literature. However, it is of course next to impossible to prove or disprove the correctness of such ecological interpretations.

A conspicuous trait in those species which can be germinated only with more or less specific and often complicated measures, is their very low percentage germination. All tested Lycoperdaceae and almost all Boletaceae species show this. Does this reluctant response reflect an intrinsically low germination power or does it simply show that the methods worked out by the mycologists are still poor substitutes for the true, natural mechanism—picklocks instead of the real key, which still remains to be found? The case of *Leccinum* is perhaps a warning against running into premature conclusions: when the effect of self mycelium was finally discovered, the attainable germination percentage rose to 50 % and higher, from the scarcely 0.1 % earlier produced with *Rhodotorula* species as inducers (Fries 1979a).

To elucidate all these specific mechanisms for spore germination one should probably try to look more into the situation in nature (Fries 1981b). *Leccinum* may also from this point of view be taken as an example. To function as an efficient distributor of the species to new habitats, other inducers than the own mycelium must be in action. However, so far extensive testings of various sorts of microorganisms isolated from forest soil have not led to the discovery of any new germination inducers more powerful than the rather inefficient *Rhodotorula* (Fries unpublished).

It is hard to imagine that such more efficient factors do not exist, which would induce germination of *Leccinum* spores in the absence of self mycelium. Occasional observations of mass germination in *Russula* species (Melin 1959, 1962; Heinemann and Gaie 1979) and in *Boletus edulis* (Orlos and Twarowska 1965) indicate that very efficient germination-inducing mechanisms may be found operating in the soil. Their nature is still unknown, however. It may be volatile exudates from roots or from micro-organisms, which as yet have escaped isolation or are difficult to cultivate *in vitro* for activity tests.

On the other hand it may be significant that many fungi which are difficult to germinate are also difficult to cultivate as vegetative mycelia derived from basidiocarp tissues. Several species of *Lactarius* can be germinated as well as cultivated, even if with difficulty (Oort 1974; Fries 1981b), whereas the *Russula* species are extremely refractory in both respects. A profitable mode of attacking the spore germination problem in *Russula*, *Cortinarius*, *Inocybe*, *Gomphidius* and other recalcitrant genera would perhaps be to improve the medium for vegetative growth. When satisfactory nutrient media have been composed, they might form a better basis also for spore germination experiments with species of these genera. The germination of the spores does not necessarily depend only on the presence of more or less specific substances of biological origin but also on delicately adjusted combinations of chemical and physical

environmental factors, conditions which may be difficult to detect and reconstruct *in vitro*.

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Communication problems in interdisciplinary research

D B O SAVILE

Biosystematics Research Institute, Agriculture Canada, Ottawa, Canada, K1A 0C6

Abstract. Communication in interdisciplinary research ranges from communication between collaborators with different interests and vocabularies, through presenting published conclusions in a form comprehensible to all potential users, to choice of a publication vehicle and facilitating information retrieval.

Keywords. Communication problems; interdisciplinary research.

1. Introduction

The enormous growth of research in the last half century has inevitably led to increased specialization by individual scientists. However, the more deeply we investigate any biological process or activity the more often we find ourselves involved in interdisciplinary research. Such involvement leads to communication problems at levels ranging from understanding between members of a research team to ultimate publication in a form that is comprehensible to all potential users of the information, in an appropriate vehicle, and with the assurance of reliable information retrieval. Before discussing the problems and their possible solutions, I shall give some examples of interdisciplinary fields. We shall see that they are often more deeply interdisciplinary than superficial observations may suggest.

2. Interdisciplinary fields

2.1 *Taxonomy and systematics*

At one time taxonomy (alpha taxonomy) was a largely isolated discipline. For vascular plants and some animal groups this is no longer true. Cytogenetics, biochemistry, ecology, biogeography, plant palynology, animal behaviour and occasionally host-parasite relationships may be involved; and it is seldom that such widely based studies can be handled by one investigator. Sooner or later numerical techniques are also likely to be involved, notably cladistic analysis for the derivation of phylogenetic trees. These mathematical techniques are described in various journals. It is notable that some important methods used by botanists appear first in *Systematic Zoology*. Thus, although papers dealing with different biological disciplines tend now to appear in specialized journals, we see that the modern systematist must be informed on many disciplines.

Numerical methods themselves present a complex problem. Being mathematical rather than biological techniques, they are equally applicable to zoological, botanical, mycological and bacteriological studies; and they may apply outside taxonomy. After Baum (1977) published a technique he was agreeably surprised to find it quoted

extensively by a psychologist in handling his experimental data. Keeping abreast of published information in numerical methods is clearly a problem.

2.2 *Pollination and plant dispersal*

Non-motile organisms depend on outside agencies for dispersal, and their study inevitably is interdisciplinary to varying degrees. Pollination is effected by a wide variety of insects (with flower morphology and scent appropriately adapted), birds, bats and wind. Ridley (1930) long ago described almost all the main methods of plant dispersal; but we keep turning up new phenomena. Dispersal by animals is very diversified, but, although we now probably know most of the methods involved, we do not always promptly recognize the method used in a particular plant or appreciate its efficiency. It is known that seeds of *Pinus* sect. *Cembrae* are dispersed only by the nutcrackers (*Nucifraga*). It has recently been shown by Lanner (1982) that this is a highly refined symbiotism. Clark's nutcracker (*N. columbiana*) distributes a pouchful of seeds in several pits over a range of up to well over 100 m. Months later, when food is scarcer, it can locate every cache, but does not remove quite all the seeds from any of them. Such a study involves ethology among other disciplines.

It was claimed by Gajewski (1957) that *Geum* subg. *Oncostylus* sect. *Neo-oncostylus* must be at least 80 million years old because its distribution in New Zealand and south-western South America is similar to that of *Nothofagus*. But *Geum* takes a group of modern rusts that make such an age inconceivable. These plants have the basal part of the stigma reflexed into a narrow hook perfectly adapted to lodge in feathers; and I suspect that they have been carried by albatrosses nesting on the vegetated cliff heads (Savile 1979b).

Dispersal by wind and water may seem too obvious to need attention; but there are various disregarded refinements. When a gentle gust strikes seeds of milkweed (*Asclepias*) or large pappose fruits of some composites they tend to scatter more upward than laterally or downward, seemingly because the pappus flops forward before the inertia of the seed is overcome, and the convex upper surface exerts lift like the upper surface of an airfoil. This interdisciplinary project will require a wind tunnel producing controlled gusts for its complete solution, not a popular idea with wind-tunnel operators. Wind-dispersal in the arctic is far more effective and common than the small numbers of winged or plumed disseminules suggest. It operates for at least eight months of the year over sea ice and wind-packed snow. Appreciation of its efficiency, clarified by some understanding of aerodynamics, transforms our understanding of arctic biogeography (Savile 1972). A former colleague once insisted that *Buddleia*, which has conspicuously modern-looking flowers, must date from mid Cretaceous because some closely related species occur in Africa and South America. At my insistence he produced the seeds of several species: all very small and with loose bladdery coats often drawn out into two tails. As sand occasionally falls on ships in mid South Atlantic it seems safe to assume that *Buddleia* seeds occasionally make the complete crossing.

Later in this paper I show that dispersal by falling water drops is a more refined process than we used to believe, and one that throws light on the ecology of the plants and fungi involved.

.3 *Animal flight*

'light studies are inevitably interdisciplinary, involving low-speed aerodynamics and ubstantial knowledge of the animals under study. My own experience in bird flight tudies impressed upon me the deplorable communication gaps in this field. Having een introduced to aircraft aerodynamics in World War II, it was natural for me to vatch the landing and take-off procedures of various birds and to note the great ariation in wing forms. With a little coaching from ornithologists I was thus able to nake substantial progress but I was isolated from the few biophysicists engaged in the eld, for, as it later turned out, they published in journals of which I had never heard. 'inally, Raspet (1960) published a paper in *Science* that solved one of my problems, the eason for pronounced sweepback in the wings of all the fastest birds (Savile 1962). Jnfortunately he died while the paper was in press, prolonging my isolation.

Study of the comparative morphology of bird wings (Savile 1957b) inevitably led me o the consideration of primitive birds. I accordingly studied the wing of *Irchaeopteryx*, with particular attention to the primaries and the highly evolved lliptical wing of advanced aerodynamic form. This interpretation was published in a eading ornithological journal (Savile 1957a) and was also quoted in my main study in *Evolution* (1957b), and I innocently assumed that the information would reach anyone oncerned with *Archaeopteryx*; but paleontologists have since written on this bird, roposing *inter alia* that the wings served not as such but as insect swatters or as shawls or protection on chilly nights. No vertebrate paleontologist known to me had seen my tudy of the wing of this competent glider. Clearly we have here a disastrous failure of ommunication.

.4 *Biomedical engineering*

'echniques are often developed in hospitals for an immediate purpose; but they may be f wider application and should be published. The more disciplines are involved and he more diverse they are, the harder it is to be sure that the publication will reach all hose who need its information.

.5 *Gas chemistry and aeronautics*

The Scottish chemist Joseph Black inflated a bladder with the recently isolated gas ıydrogen, simply to demonstrate its lightness to some friends. He had no thoughts of ıseful applications. However, the Montgolfier brothers, who had pioneered with hot-ıir balloons, stumbled on an account of Black's experiments and practical balloon ravel was promptly developed. The communication in this case was purely accidental Scott 1984).

.6 *Genetic engineering*

am not involved in this new biological field, but it clearly involves various disciplines. ndeed the borrowing of organelles and genetic material seems to have been 'undamental in the evolution of the eukaryotes. Among persistent symbiotic ıssociations such as lichens, or the rust fungi and their host plants, it is quite possible hat natural genetic engineering has been operating nearly since the origin of land

plants. This field may prove difficult to study; and, because it may involve new concepts and several disciplines, it may breed new terminologies that will add to the communication problem.

3. Communication problems and possible answers

Communication problems occur at several levels.

3.1 *Manner of presentation*

An interdisciplinary paper is inevitably directed at readers in more than one field. First we must present our data and conclusions clearly and forcefully to people in our own disciplines; but, to reach readers in other disciplines, technical terms should whenever possible be replaced by plain language. When such terms are needed, for brevity or precision, they should be explained. Complex methods, *e.g.* of mathematical analysis, can often be explained as to function only, and references given for the reader concerned with details. If we overwhelm non-mathematical biologists with all the details we simply lose them. If all potential readers are biologists a lucid presentation may be relatively simple; but when a physical science is involved particular care must be devoted to presenting all parts clearly.

Workers in all groups of organisms have their own terminologies, which they use casually among themselves but which require some explanation for wider comprehension. For example, Dr Bernard Baum and I recently concluded a study on the co-evolution of the genera of Triticeae and their rusts. It was inevitably complex to present and it went through several versions until it seemed clear; indeed it *was* clear to us but only because we had lived with it for many months. When a fellow botanist with limited knowledge of either the Triticeae or rust fungi read it she showed the need for clarification at several points, but more importantly she insisted on major shifts and regrouping of various sections, which made easier reading and gave much more emphasis to some of the findings. Many reviewers will not read all parts of a paper equally critically. Such perceptive and analytical reviewers are not common, but for complex papers they are invaluable.

As scientists we are trained to present our work as briefly as is possible, consistent with clarity; avoid duplicate or overlapping publication; and above all observe the utmost modesty in presenting our achievements. In interdisciplinary papers these rules may have to be relaxed somewhat if readers in marginal disciplines are to grasp our conclusions. Overmodesty in presenting our findings makes them obscure to such readers. There is no law that says we must be so dull as to put our readers to sleep. If a whimsical phrase helps to put an idea across, by all means use it: not only may it keep your reader alert enough to realize that you have some help for him, but he will probably remember it.

After the paper is submitted for publication the second round of reviews starts, over which the authors seldom have any control. Reviewers vary greatly in quality and experience. However, any competent reviewers can usually help the authors to clarify a few points, simply because they have been isolated from the work and have a new perspective. However, acceptance by reviewers does not guarantee comprehension by all potential users of an interdisciplinary paper; and publication does not necessarily mean that we have succeeded in our study.

Occasionally a piece of work is lucidly presented and appropriately published but attracts little notice—it seems to be ahead of its time and is overlooked for years. Mendel's work was well distributed, going, as Mayr (1982) points out, to at least 115 institutions (a large number for 1866) including at least two where Darwin might have seen it, and to various individuals. Apparently no one saw the significance of it until the growth of cytology in the next 25 years had prepared the ground. Yet it could have provided Darwin with the evidence that he needed for particulate inheritance. Today we can only speculate as to whether a different method of presentation, appropriate to the 1860's, could have convinced contemporary biologists of the value of Mendel's work. A more recent parallel is the delayed recognition of Barbara McClintock's pioneering work in maize genetics, which demonstrated the occurrence of genetic transposition. Although the correctness of her conclusions was realized by other maize geneticists, it was not generally accepted until the same process was found in bacteria. It is at least satisfying that she was still with us 40 years later to receive the Nobel Prize. There is no obvious excuse for this second example of delayed acceptance except mental rigidity, for the work was well and widely presented. I am unqualified to judge whether it could have been even better presented.

3.2 *Communication between team members*

The team approach is widely hailed as the answer to interdisciplinary problems, but, vital as it often is, it is not an automatic solution. At its worst the team is a group of specialists who remind us of the six blind men trying to describe an elephant: they individually likened it to a wall, a tree, a fan, a snake, a spear and a rope. For full and prompt success at least one team member must walk all round the elephant. By learning something of each others' work, terminologies and applications the members learn to see the problem as a whole.

The first problem is thus communication and comprehension within the team. A corollary is that, within practical limits, the smaller the team the better their understanding. At this point I am reminded of an article written by some American scientist, burdened with them in World War II, on the mathematics of committees. He concluded that the optimum committee size was one, because in any event the chairman had to do all the work. Occasionally that approach works well in biological problems: the biologist happens to have adequate knowledge of the second discipline; and, communicating freely with himself, solves the problem before two specialists could appreciate that one exists. However, a team is often needed and, the more diverse the disciplines, the more careful must the members be to make their terms, methods and aims clear to each other.

Having done a few individual interdisciplinary studies, notably in bird flight and in arctic seed dispersal, for which my war-induced elementary aerodynamics had been adequate, I was unprepared for the problems that can occur in a joint project. Following the lead of Brodie (1951, 1955) I could recognize and admire splash dispersal devices, both splash-cup and springboard: it was quite straightforward—raindrops do it. But I was haunted by the recollection of arctic sedge meadows with profuse colonies of *Chrysosplenium rosendahlii* in a region where rain is usually fog or drizzle often slanted by wind. Then Brodie (1975) recorded a bird's nest fungus, *Cyathus olla*, under shrubs in the Peruvian desert where fog is the only precipitation. Clearly our ideas needed revision. Anyone who has used an umbrella knows that drops

are larger (louder) under a tree than between trees; but how much larger and how much more effective? Nobody seemed to know. I did find some data on drag coefficients and terminal velocities of drops in vertical wind tunnels, but my atrophied mathematics could not let me go further. I was mercifully put in touch with Dr. Henry Hayhoe, a mathematician in our Agrometeorology Section, who derived an equation allowing us to find the velocity of a drop of given size at any distance of fall. But it was not as simple as that: I could not follow the mathematical methodology; and at first the mathematician, unfamiliar with the plants, could not see just what I wanted. However, when we finally plotted the results our problem vanished; a drop from the canopy has a higher momentum at less than 0.5 m fall than even an unusually large raindrop at terminal velocity (Savile and Hayhoe 1978; Savile 1979a).

Similarly, when Dr Baum and I undertook our cladistical study of the rusts of Triticeae and their hosts neither realized what was in store for us. He was repeatedly misled by my discussions of the rusts, which were simply names to him; and, as I had not been involved in a numerical study and could not understand the language, he needed great patience in explaining what we were doing. Thus much time was lost through our communication problems.

I also recall that, when a local hospital appointed their first engineer to the professional staff, his first months were most frustrating because most staff members could not believe that he could help them or even that they needed any help. There was simply no effective communication. The situation improved radically once they began to understand each other.

3.3 *Method of publication*

The choice of a journal is particularly important in interdisciplinary studies. Ideally the paper should appear in an interdisciplinary journal. Papers dealing with interactions between fungi and plants I have usually published in *Canadian Journal of Botany*. However, when I submitted a paper on the rusts of Cheloneae, which also included the documentation of a rust jumping from *Penstemon* to *Pedicularis*, finally explaining why it is that rusts and their host plants reflect each others' ages, a horrified reviewer insisted that this nasty theoretical appendix (perhaps my most important contribution to biology) be removed. Despite my protests the editor concurred, and the paper was finally published in *Nova Hedwigia*, whose editor appreciated it. Unfortunately this journal covers all cryptogams but not flowering plants. It was only after the jump mechanism was redescribed in a symposium paper in *Quarterly Review of Biology* that I was flooded with reprint requests.

Papers embracing botany and zoology (*e.g.* pollination biology), or botany and physics present a special problem now that such general journals as *Nature* and *Science* are so crowded with specialized papers, rarely on organismal biology, that they usually will not publish an interdisciplinary one. Thus, we have effectively lost what were interdisciplinary scientific newspapers a generation ago.

If the paper is short it may be most practical to publish it in a journal of whichever field seems more important, and send reprints to all the people in the second field whom we expect to be interested. With a longer paper cost can be kept down by sending only copies of the abstract or particular pages to prospective users in the secondary discipline. The obvious weakness in this method is that there are bound to be prospective users whom we do not know. Following this method Dr Hayhoe and I put

our note on drop momentum in *Canadian Journal of Botany*, among whose readers most interest could be expected, and Dr Hayhoe sent copies to meteorologists. A continuing trickle of requests, largely from plant pathologists, seems to justify our action. To make the information available to park naturalists who like to have something for visitors on wet days, and amateur botanists, I also put an illustrated note in a west coast magazine (Savile 1979a).

Indexing and abstracting programs and publications are increasingly important for information retrieval in many disciplines. In theory they should alleviate our problems in interdisciplinary communication; but, although they may be increasingly useful in the future, they seem to be of limited use now, and I have the feeling that some indexing is closely restricted to a single discipline. After I was asked to review the use of fungi as aids in plant taxonomy (Savile 1979b) one of our librarians undertook a computer search of titles in the preceding ten years; but the only truly pertinent titles found were two of my own. Accordingly I used the time-honoured grapevine system (sending circular letters to many colleagues), supplemented by contacts with the contributors to a symposium on nearly the same theme (Hedberg 1979).

Clearly we must give indexers and abstracters all possible help. Keywords are a distinct help if well chosen, but not all journals use them, and indexers will not necessarily use all of them. A good title is extremely important, since it must be cited in full. If a taxonomic paper on a group of parasitic fungi deals also with ecology, biogeography and host relationship, it is advisable to include these words even if the title exceeds a line of type. The literature searcher may not see the abstract. Also the expanded title may allow a shortened abstract.

Occasionally an attempt at communicating a multidisciplinary study to a diverse readership is foiled by editorial action. Near the completion of a study of evolutionary trends in the rust fungi (Savile 1976), Dr. Kris Pirozynski and I stumbled on the evident mimicry by *Ravenelia* teliospores of the compound pollen grains of *Acacia*, the main host of this rust genus. Mimosoid pollen sheds freely on the foliage. It is suspected that bees (probably in large part stingless bees of the genus *Trigona,* which do sweep up pollen) collect the pollen and the geometrically similar rust spores, occasionally flying to another relatively distant tree with their mixed load. Here we had: an example of mimicry involving members of three kingdoms, plants, fungi and insects; the explanation of the seemingly ridiculously large compound teliospores of *Ravenelia*; and, as a comparative study of a long series of mimosoid pollen showed, an indication that their compounding, thickened exine and pigmented exine were adaptations against aridity. The brief report was sent to general journal, with, following editorial instructions, a selection of competent reviewers in the fields of tropical botany, plant evolution, tropical rusts, and pollination biology. None of these authorities were consulted, but a disparaging reviewer suggested it be put in a palynology newsletter—surely a perfect burial ground for an interdisciplinary topic. The editor rejected it out of hand as being speculative, a characteristic of all hypotheses. Perhaps he blamed us for not being there throughout mid Cretaceous recording our observations. The full story remains to be worked out, because it is difficult to contact possible observers without a widely available account.

There is no complete or simple solution of these communication problems. I think some pressure should be applied to indexing and abstracting organs to treat interdisciplinary reports as such, rather than listing them under only one discipline. It would be a great help if at least one general journal would accept the responsibility for

publishing brief interdisciplinary reports, perhaps with references to fuller accounts in more specialized journals. Finally, if such efforts fail some attempt might be made to set up an indexing organ purely for interdisciplinary papers; authors would submit copies of the paper with appropriate subject headings, and involved scientists would use the index in their literature searches.

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Plant cell physiology (1934–84): Recollections and reflections

F C STEWARD
Professor Emeritus, Cornell University, Ithaca, New York, USA
Mailing address: 1612, Inglewood Drive, Charlottesville, Virginia 22901, USA

Keywords. Plant cell physiology; metabolic machines; osmotic machines; ion accumulation; growth promoting substances; trace elements; solute composition, development.

1. Prologue

This essay relates largely to the past; this prologue sets it in the context of the present.

An invitation to contribute to the golden jubilee celebrations of the Academy founded by Sir C. V. Raman, Nobel Laureate in physics—is not to be treated lightly. If the responsibility is accepted how should it be discharged? A piece of original research dedicated to this end should not be trivial and merely to recapitulate would also be unworthy. This essay therefore will not stress specific contributions, with their cited observations in support—to do so would merely invite becoming submerged in a mass of details. A different aim is to view plant physiology more comprehensively and to see how it has fared in a period of great advances in physical science. This is best done through salient topics with which the author was in touch throughout and which invariably distinguish living plants from inanimate systems. Although the account is brief its scope is broad.

As plant physiology emerged into the twentieth century the trend was already set upon applications of a rational system of chemistry on the one hand and on developments from the cell theory on the other. The main focus today is upon causal physicochemical explanations of vital phenomena. The period in question (1934–1984) was dominated by technical advances in plant biochemistry, intermediary metabolism, enzymology, and the philosophy of genetics and molecular biology. The prevalent trend was toward reductionism, *i.e.* the attack upon problems and systems reduced to units such that they may be separately comprehended. This approach may seem to be vindicated by the wealth of information that it has yielded. But how far does the summation of that information comprehend the life of the organisms in question?

The reactions of intermediary metabolism sponsored by innumerable enzymes, each seemingly gene-controlled, now seem overwhelming. Any modern text that summarises biochemistry readily overflows with charts that link reactions into schemata and schemata into concepts, whether of photosynthesis and respiration as they involve carbohydrates and organic acid metabolism or the biochemistry of nitrogen compounds for the amino acids and protein metabolism synthesis and breakdown. The specific reactions and systems that relate to other broad areas of biochemistry have also proliferated. But most noteworthy is the recognition of phosphate bond energy as the energy currency through which specific energy requiring transactions are negotiated as it has pervaded all of biochemistry and physiology.

The combined resources of radioactive labelling of substrates and of various forms of chromatography (now very far advanced from the first efforts in these directions early in the post World War II period) have produced volumes of data (often described and interpreted by scientific art forms as in *Scientific American*). These now convey an air of certainty to events that are presumed to occur at templates, on membrane surfaces and in organelles.

Membrane phenomena; and what hitherto seemed to be the relatively simple events of their permeability to water and to solutes have been overtaken by concepts mathematical and mechanistic, that at the turn of the century and in the wake of the classical period of cell physiology would have been inconceivable.

But not withstanding the impressive scope of all these and other events the over-riding considerations still involve the organisations in nature that make them feasible and the forces and energy relations that integrate them into a coherent working whole.

The cell theory and the first thoughts about protoplasm and the cell nucleus awaited the slow but nevertheless dramatic rise of submicroscopic morphology. This was later interpreted by electron microscopy and its satellite techniques. The picture that emerged was of an internal universe of complexity in which the vital events occur in cells and their organelles. So the over-riding questions that have still to be faced concern the means by which all the attributes with which cells are endowed are motivated to proceed harmoniously in time and space and compatibly with an evolutionary history, on the one hand, and an inherited developmental plan, on the other.

But all these questions and interpretations at the cellular level must also be comprehensible at the level at which cells are organised into tissues and organs and, in higher plants especially into the growing regions from which they derive. Morphology and embryology, which in their first descriptive phases were predominant areas of biology were later overwhelmed by physiology, biochemistry and nutrition etc, have now come again into their own. It is through morphology at all levels, from the molecular to the subcellular, and in the growing regions of plants that milieux are created in which events are shaped that otherwise are difficult to comprehend in physical and chemical terms.

There is a paradox here that may be illuminated by the following story. In the 1920's, as an undergraduate in chemistry at Leeds, the writer came into contact with an able laboratory demonstrator, who shall remain anonymous, but who was then fresh from Oxford. His first work had been in the discipline of crystallography. However he had forsaken this subject because, as he said, it was one in which all was seemingly known, in the sense that all of the feasible crystallographic forms were then known to occur. So he left crystallography for spectroscopy which he deemed closer to the frontiers of science. But whatever the wisdom or otherwise of that choice, crystallography was about to take on a new and unexpected scope. The repeating regularity of molecular structures in space, whether in polysaccharides (as in cellulose) or in nitrogen compounds (as in proteins and the ribonucleic acids), essentially in their structures display the features of the crystallographic state. They also extend its interpretations into more dynamic spheres which have brought this type of physical structure of matter close to the very mysteries of life.

But also in the 1920's and early 1930's one used to challenge students with the numerous reasons why nature built so much complexity upon the compounds of carbon with carbon and also with nitrogen. But why not silicon? Little did we then know how much nature could harness the storage of usable information and energy,

into the geometry of arrangements of carbon with nitrogen and phosphorus, while it left to silicon the basis of the chemistry of minerals. Strangely perhaps, we then knew so little about the near miraculous properties now known to flow from the recoverable storage of useful information on silicon chips.

Thus we should now look beyond the present glut of information about plants, their metabolism and chemical working to seek in their organisation at all levels (from molecules to morphology) the clues to their success without violating the principles of matter and energy as they operate in the material universe. The 'message' could stop here, but it will be pardonable to present some illuminating sequences—known to the writer through personal experience—that illustrate how this general philosophy came about.

One may begin with problems of plant cells as they absorb and hold water and solutes from the ambient media. In plants as indeed in animals water is the most abundant natural constituent. In fact nature imposes restrictions upon the water of the animate world, which distinguish it from the inanimate world. But plants in the waters of the earth, or in their terrestrial habitats create and maintain distinctive compositions which set them apart from their environments. This is no mean physical achievement for it involves the organisation we call life and, with dissolution and death, uniform physical equilibria return. Problems of membranes at boundary surfaces inevitably arise to know how far their behaviour is intelligible in terms of familiar physicochemical principles. Viewed as chemical working machines the cells of plants in their metabolism have developed and use a great and growing array of biochemical substances and systems so that, over the 50 years in question, a body of biochemical knowledge inconceivable at the beginning of Raman's career (or mine) has emerged. Also, since metabolism works, perforce, according to an inherited plan genetic transmission of the metabolic information and the mystery of its regulatory control has loomed large in the period.

Whereas in the period physicists and astronomers have presented a truly awe inspiring picture of the material universe, biologists may now add to cosmology their own vision of complexity in an internal biological universe (inner space vs outer space) that must nevertheless also operate according to a physical plan. This plan, however, contrasts with the cosmic one which operates over the vast distance of time and space for it is comprehended within the sub-cellular and even molecular dimensions within cells and their organelles. But cells are also organised externally into tissues and organs and, in higher plants, they arise from growing regions. Thus within the plant body other challenging problems of organisation and integration arise. As a higher plant grows and develops it creates a morphology that provides the internal environment in which metabolising cells function in many organ specific ways. In turn morphology is often modulated by environments. If science is viewed as an adventurous journey into the unknown, as indeed it has become in the physical universe, then the challenges of plant cells and plant physiology are as arresting, though perhaps more attainable, as are those of astronomy and cosmology.

Time was when the style and title of natural philosophy could embrace all the enquiries being made by man about his world. As knowledge increased specialisation ensued. Mathematics, astronomy, agriculture, medicine, the natural sciences, chemistry, physics with botany, zoology and bacteriology and later genetics appeared as distinctive disciplines. Thenceforward the process of specialisation and reduction in the scope of a field of enquiry to be covered proceeded. More and more was expected about

less and less. As exciting new developments occurred at the overlapping margins of the more traditional disciplines they gained recognition and support by labels under which they flourished for a time. Cytology, general physiology, cell physiology, general cytology, biochemical cytology, dynamic biochemistry, enzymology, biochemical genetics, molecular biology, biological and genetic engineering and so on. But the loss of the erstwhile simplicity of *subjects* was more than rivalled in the terminology of *substances* and systems, involved in natural processes. Acronyms, often meaningless without a glossary multiplied and the modern terminology of complex organic structures and of enzymes, is a linguistic nightmare. Though meant to achieve precision it certainly has complicated communication to the point where one wonders whether truth as properly understood, needs to be so 'unrecognisable'.

Often newly identified areas of specialisation acquire their identity through an advance made in laboratory technique or in instrumentation. As each new area is pursued to the limit it reaches the point where further understanding demands that it be seen in a wider context. Thus the first gains achieved by specialisation and reductionism need then to be balanced by, or set in the perspective of, an enlightened holism. Some landmarks along this route may now be noted.

2. Towards plant cells as metabolic and osmotic machines

Cell physiology, an off-shoot from the cell theory in the mid 19th century began to pay plant physiological dividends in the late 19th century as the foundations of the subject were laid and modern plant physiology developed through the first quarter of the 20th century. But, even as early as this, it was evident that physical and botanical science were to be joint beneficiaries of the new knowledge. The names of de Vries, Pfeffer, Hoffmeister, van t' Hoff and Arrhenius, became as familiar in the 1920's to students of physical chemistry and of botany as indeed were the terms osmosis, osmotic pressure, isotonic solutions, plasmolysis etc. The study of the "attraction for water" of solutions, whether they were bounded by "semi-permeable membranes" supported by porous pots or the membranes of living cells, gave rise to a theory of solution that brought the gas laws and gas pressure into harmony with what came to be called osmotic pressure. These relationships were made intelligible through the colligative properties of solutes that operate by reducing the vapour pressure (or the escaping tendency) of the pure solvent. Once seen, these relationships, based on strict thermodynamic proof, are powerful testimony to the role of water in the physiology of plants which need not be obscured by the modern craving for frequently revised terminology and for elaborated symbolism (whereas *P* for osmotic pressure earlier sufficed the greek alphabet is now fully exploited from ϵ to ψ!).

Plant cells as osmotic systems, bounded by membranes, invited studies and speculation upon the nature of these membranes and of their permeability properties but also aroused speculation upon how cells acquire their internal solute concentrations, especially where these are heavily composed of inorganic ions that only occur in the waters of the earth at low concentrations and which as charged ions, do not readily enter the cells across their boundary membranes. Concurrently much was being learned about the nature properties and structure of limiting membranes at the surfaces between immiscible phases (of which the water/air surface is a specific case in point). Substances that reduce the interfacial tension tend to accumulate there and their

behaviour in oriented thin films like "gases in two dimensions" when expanded or like liquids, even solids, when compressed allowed them to recapitulate in films many of the familiar properties of matter in bulk. All this brought a great body of knowledge about molecularly oriented thin films to bear and to generate speculation upon the role of membranes in cells. But questions arose whether such membranes play in cells a passive physical role or a more active and dynamic one by virtue of the fact that they are an intimate part of a system that is essentially alive.

3. The accumulation of ions: osmotic work by cells

In 1929 a happy circumstance brought together Hoagland (of the Division of Plant Nutrition at Berkeley, California) and the writer (then a Rockefeller Foundation Fellow on leave from Leeds, University in England). Prior work in Leeds and at Cornell University had focussed attention on the difficulty with which solutes (especially ions) traversed isolated membranes composed of plant cells and the great tenacity with which slices of such tissue retain their solutes, even against water under aseptic conditions. In the mid 1920's, Hoagland, directed to *Nitella* by Setchell, an algologist, discovered that the *Nitella* cells could "accumulate" bromide ions from very dilute solutions in pond water in the light but not so in the dark. Hoagland very perceptively, recognised that this was a case of cells deriving energy from their own metabolism, fuelled by photosynthesis, to drive a non-equilibrium movement of ions. Hoagland used *Nitella* as a model system but he was ultimately interested in roots and their relations to the very dilute soil solutions from which they also accumulate ions. To find out the conditions under which *thin* slices of potato tuber tissue, without any *direct* access to photosynthesis, could also accumulate bromide led to the important discovery that these cells harness their aerobic respiration to the process. To enable the potato cells to perform the act of ion accumulation they responded to the necessary oxygen supply in well aerated solutions and they liberated carbon dioxide from stored starch which was hydrolysed as the cells were activated in response to aeration (This work anticipated by many years the widespread recognition of "active transfers" of ions and solutes driven by metabolism).

From this point on (1929 to 1934) progress was made both in Leeds and in Berkeley along lines that foretold much of the later developments in this field, developments which illustrated how dependent plant cell physiology was to become upon physical and biochemistry, on the one hand, and upon a perceptive insight into the role of cells on the other; even ultimately on the behaviour of cells being stimulated to grow and so to perform cell physiological functions that they otherwise could not achieve.

4. Towards a synthesis of form composition and energy

In retrospect the year 1934 marks a stage in this work and in its philosophy. After a further period of work in Hoagland's laboratory (1933–34) and also periods at the Dry Tortugas to study *Valonia* in its habitats an opportunity was seized to take stock. This occurred in an interlude on the Sands of Monterey, California.

Using the thin discs of potato tuber methods had been worked out to study the effects of different variables on the salt and water relations of the tissue and the concurrent effects on its metabolism as they were reflected in the respired carbon-

dioxide and the conversion of soluble non protein-N to insoluble protein-N. Meanwhile Hoagland had perfected the use of excised root systems of barley so grown that they had a vicarious ability for short periods to absorb large amounts of KBr ions while, concomittantly, they emitted CO_2 as organic acids declined. The two systems had illuminating points of both similarity and difference.

It was already known that the cells of the potato discs could in moist air alone, retain their viability for long periods and even draw upon their stored organic nitrogen (amino acids) to nourish the cells when they eventually divided in the formation of a phellogen. (In fact the potato cells were autotrophic for substances that were later to be recognised as necessary for other systems of mature cells to undergo cell division). In consequence the potato cells would retain their active metabolism and ability to accumulate ions (K and Br) for relatively long periods. Moreover different conditions which affected the tissue did so in ways that also tended to influence, even to suppress, the salt intake and also the synthesis of alcohol insoluble-N (protein).

But the cells of the barley roots were different. Shorn of their shoots and without any ability for secondary growth the barley roots did not rely upon new growth or protein metabolism to foster salt uptake but replaced organic solutes accumulated by those cells during their earlier development under conditions such that their normal complement of salts was trasferred to the shoot in exchange for high sugar and organic acid content sent to the roots. When excised these barley roots therefore enjoyed a brief period as "as high sugar low salt"-roots which accumulate $\dot{K}\cdot Br^-$ ions in exchange for solutes already accumulated during their attachment to the shoot and without a surge of new protein synthesis of new cells.

Thus, at this period, general schemata were drawn up that related the ability of different systems to accumulate ions using the energy of metabolism to do work and with the duration and extent of the absorption process being related to the ability of the cells for new growth and to the prior nutrition they had received.

Premature attempts were made at Berkeley to see whether the use of metabolites to produce metabolically useful energy could be detected by calorimetric methods to measure energy output and to erect balance sheets to trace the uses made of the endogenous substrates. Although this work showed how such balance sheets could be prepared they did not, and could not, identify specifically a measurable moiety of metabolism and energy that could be allocated solely to work done in accumulating salts. The picture was, and is, that the conditions established the status of an on going active metabolism in the cells and, once this was established, the cells took the process of accumulating salts in stride along with other concomittant events (*e.g.* protein synthesis etc.).

It was recognised, however, that further clues to understanding the continuing process of ions absorption in the potato and similar systems were needed and especially its relation to further growth in the cells and to the part played by the nitrogen compounds. With the methodology then available there was just time before the outbreak of World War II to trace out the effects of different variables on the endogenous nitrogen compounds and the conversion of stored starch to carbohydrates to CO_2 etc and their relations to bromide accumulation. All of this contributed to what was termed the biochemical background against which ion accumulations occurred. All this was very much more than a problem related solely to membranes. (The results were written-up sent from London to Hoagland who communicated them to *Plant Physiology* even during the war). But the intimate relations of cell growth and protein

metabolism to ion accumulation had to wait until after the end of World War II. As this story unfolded it showed again how intimately and indissolubly the problems of cell physiology became integrated with cell growth and development and can only be fully understood when this is appreciated. Indeed even in *Valonias* and *Nitella* their normal sap composition in their habitats had reflected this fact.

5. The aftermath of war: a new approach

A fresh start in research provided the stimulus to seek a more versatile system than even the thin discs of potato tuber. The aim was to seek a system amenable to work under completely aseptic conditions, in which the cells could be placed for long periods under conditions conducive to their most rapid growth by cell multiplication and/or enlargement and, alternatively, under conditions of maintained quiescence with minimal growth. All this required that external media should contain nutrients, organic and inorganic, as well as any special exogenous stimuli. To achieve these ends attention was turned to the so-called tissue culture methods then in use by White and Gautheret, although, in point of fact the *modus operandi* finally adopted for secondary carrot phloem explants was entirely redesigned to fit the needs of these investigations.

At that time the field of growth promoting substances (which has since proliferated enormously) was still dominated by the almost exclusive attention paid to IAA as the universal auxin. In the outcome the events (circa 1950) which transformed the approach to problems of salts and water in plant cells also impinged upon many other areas of enquiry. This was especially true for the following problem areas which were profoundly affected.

The growth promoting substances involved in the induction of growth in otherwise mature or quiescent cells.

The nitrogen and protein metabolism of plants, especially the composition of the endogeneous, nitrogen compounds and their mobilisation during growth induction and protein synthesis.

The aseptic culture of isolated explants of plant tissue and especially as this is carried out in liquid media.

The ability to produce and culture free somatic cells.

The totipotency of free cells and their ability to behave as somatic embryos and thence to produce clonal populations of plantlets.

Finally the interest in the organ specific solute compositions of cells during normal ontogeny, was revived and especially as this may be subject to environmental effects upon morphogenesis; effects that originate in the growing regions.

Because so many inter-related topics were affected in these ways the full impact of all cannot be separately stressed here. It can be seen, however, by reference to other publications. A general point may, however, be made.

Whenever it becomes the vogue, even fashion, to attack problems in a prescribed, acceptable way real progress may become deadlocked. This undoubtedly occurred in the belief that many problems should be attacked without encountering the "complications of growth". But what is plant physiology without "the complications of growth"?

The steadfast disregard over many years of cell multiplication as the first and all-

important facet of growth, that it so clearly is, while the role of auxin (IAA), at stages in the coleoptiles of grasses that involve only cell enlargement, was termed *the* growth hormone, was clearly unfortunate.

Also the failure to perfect liquid media for the aseptic culture of angiosperm cells thus confining this technique for too long to random proliferations on relatively large explants on agar was not conducive to rapid progress. Furthermore, to restrict these media to none other than well known constituents and fully "defined" substances discouraged investigators for years from searching for the unknown systems that cause rapid cell multiplication.

6. Cells in culture and in situ: Their physiology as they grow

But, even so, when a system based on culture of carrot cells that could be placed alternatively under conditions conducive to rapid cell multiplication or a relative quiescence was clearly in sight, its exploitation in the problems of solutes in cells was deferred. Deferred, in fact until the system and methods for its full analysis were developed. When, in fact this position was reached and its wide implications were realized it also prompted a return to the problems of growth as they are seen in the growing regions. For this culture of plants (potato and carrot), under the controlled conditions by then available in growth chambers, were exploited so carrying the knowledge gained about cells in culture to their behaviour *in situ* during normal development. This emphasises a hitherto much neglected area of plant physiology namely the extent to which metabolism, form and composition of plants with their genetic composition already defined, are affected by environmental factors as they interact both diurnally and seasonally.

But one should return to the main theme of research into the ability of cells to accumulate solutes. This involves relating the process in question to cells as they grow and develop. The mature understanding of these problems emerged from the use of the system of cultured cells which consisted of small, uniform, cylindrical tissue explants of carrot root secondary phloem cut far enough from the cambium so that *in situ* they would not divide again. Whereas aseptically (whether in water dilute salts or even nutrient solutions) they merely enlarge somewhat their rapid growth in culture by cell multiplication may nevertheless be chemically induced.

The role of plant growth hormones, or growth regulatory substances was long dominated by the analogy with hormones in the animal body and their specific "actions at a distance". This emerged from the classical role of IAA as it exerted its effects primarily upon cell enlargement as in the oat coleoptile. But the most powerful "Growth promoting effects" on the cells such as those of carrot root, otherwise unlimited by endogenous or exogenous nutrients are those that first promote cell multiplication and thereafter cell enlargement in balance. To promote these effects recourse was made not to already "defined" substances, but to substances from the environments of zygotes as in the contents of the coconut (its liquid endosperm or coconut water or milk), of the immature fruits of *Aesculus* or even extracts of immature grains of corn (*Zea mays*). Under appropriate conditions that have been described elsewhere these stimuli cause the carrot explants to grow rapidly and, as they do so, they acquire solutes (organic and inorganic) in their cells.

The first solutes to be absorbed and secreted internally into the cultured cells as they

multiply are organic solutes (sugars and organic acids, the latter electrochemically balanced by $\dot{K}$ which is, however, not yet accumulated to high concentrations or accompanied by Cl^-). In fact it is later when cell multiplication subsides and enlargement intervenes that the endogenous organic solutes can be replaced with salts (preferentially of $\dot{K}$ although now it may be accompanied by Cl^- and even some $\dot{Na}$). In fact in this active developmental state cells may be induced *reversibly* to replace organic solutes previously absorbed with inorganic salts exogenously supplied.

In other words the cellular control is primarily over the activity of water in the cells with enlarging vacuoles and not with ionised salts *per se*. Thus investigators have been so preoccupied with the electrogenic events at membrane surfaces in pre-formed mature cells and not primarily with the *de novo* accumulations of solutes that the significance of *total* solutes and their colligative properties upon internal water have been obscured.

Nevertheless, when one moves from cells in aseptic culture to cells as they develop *in situ* they accumulate solutes in accordance with their morphological setting and the environmental conditions of length of day or night or the temperatures by day or night. Potato cells in developing leaves or in tubers acquire solutes according to the environmental conditions that regulate development. All that need be said here is that the variables that affect metabolism in these developing systems also affect the total solutes they contain and their relative composition in terms of organic and inorganic solutes *in situ* in the intact plant body. This is true of cells of both potato and carrot plant during development.

However, the belated investigation of problems of solute composition in cells under aseptic culture led to other unexpected discoveries.

7. Roles of growth promoting substances and trace elements

The study of the naturally occurring agents that induce the growth of explants of carrot phloem in aseptic culture produced two types of unexpected conclusions. These relate first to the nature of the growth stimulants *per se* and also to their interactions with trace elements.

The most effective stimuli for growth induction of carrot cells were found in such fluids as coconut milk or water; similar fluids or extracts from immature fruits of *Aesculus* (particularly *A. woerlitzensis*) or in immature corn (*Zea mays*) grains. These were divisible into different systems that could be separately assayed. Two such systems differed in that they reacted synergistically in the presence of either IAA or with certain other naturally occurring constituents of which myo-inositol was the prime example. These partial growth promoting systems, designated I and II comprised their respective active cell division fractions (AF_1 or AF_2) become effective in presence of their respective synergists (AF_1 + inositol) or (AF_2 + IAA) and they may also act best in presence of casein hydrolysate. $A'F_1$ has been best obtained from immature *Aesculus* fruits and AF_2 from coconut has been found to be replaceable by zeatin. However, with or without casein hydrolysate these systems only produce their full effects under an appropriately balanced trace element regime. While Fe is paramount in the relation of the trace elements, to the growth stimulus due to the exogenous growth factors the tissue also responds to trace element regimes involving Fe, Mn and Mo. To work out and present a complete picture of all these interactions between components of growth

factor systems and with trace elements as they affect the growth and composition of carrot cultures proved to be a formidable task. (It has been described as far as possible in two series of papers, the one published in the *Annals of Botany*, the other in *Planta*). However, in addition to the varied responses of the tissue to the growth factor systems and to trace elements a most far-reaching and unexpected one on salt composition was observed and described.

The consistent effect is that cultured carrot cells as indeed many other angiosperms accumulate more $\dot{K}$ than $\dot{Na}$—so much so that often $\dot{K}$ very greatly predominates over $\dot{Na}$. However, it was found that under the unbalanced stimulus of certain adenyl cytokinins; normally a constituent of carrot growth factor system II, this relationship was so drastically changed that $\dot{Na}$ even predominated over $\dot{K}$ in the cultured cells. In a study of homologues of zeatin (applied without IAA) it was found that their maximum effect on the Na/K content of the cultured cells occurred when the zeatin side chain was $-(CH_2)_4 \cdot H$. But in the presence of IAA the number of cells increased and their Na/K ratio was, as usual, low. This is a very far-reaching effect of a growth factor system on the ionic composition of the cells. It would, however, be tedious to enumerate here all the ways in which external variables affect not only the growth but also the composition of the cultured cells. The overall conclusions may be summarized as follows.

8. The final modulation of solute composition

After cells are endowed with their unique genetic inheritance their behaviour with respect to metabolism and to their solute contents is mediated during development in various ways. First their morphological environment has an over-riding effect i.e. whether cells develop in one tissue or organ or in another. Second, after the cells develop in a pre-determined location (*e.g.* in leaf or tuber in potato plants, in secondary phloem in storage roots of carrot or elsewhere) they will still respond during development to over-riding effects of external physical (*i.e.* environmental) variables in which length of day (or night) and temperature by day or night interact. One may extrapolate from the studies on cells in aseptic culture and expect that if all the possible effects of mineral and trace elements nutrition together with the effects of environments on the composition of cells in storage organs and leaves were fully investigated their range and scope would be very large indeed. Conversely to select arbitrarily contrasted pairs of these variables for separate investigation and publication would produce fragments of too dubious value from which to draw over-all conclusions. However, the short answer is that the biochemical composition and the salt and water content of cells as they develop *in situ* and respond during ontogeny is a very complex question which is unlikely to be amenable to preconceived mathematical or electrogenic analysis. It must be subject to an essentially obvious conclusion. Plants in their environments develop as physical systems that enable them to respond in their cells to interacting external variables in ways that give them the physical stability that they so clearly possess and this physical stability seems to be mediated ultimately through the properties of water.

9. Problems: physical and biological

Paramount problems still seem to be the following. How do plants endowed with their unique genetic inheritance respond during development so that, *in toto* (though

perhaps not necessarily gene by gene) they achieve the remarkable degree of response and stability in their form, composition and behaviour? Also as cell tissue cultures have shown, ontogeny and diversity go hand in hand even while this is still compatible with a maintained totipotency at the somatic cell level as shown by the events of somatic embryogenesis in culture. But the means by which the information carried by genes is recalled for expression in all this remains a physiological mystery.

However, to allow for inherited variations to occur through sexual reproduction, cells at meiosis depart from the diploid state, and in plants, they may even continue to develop in this state as gemetophytes. Although haploidy has been observed to occur and is so documented for carrot cells in culture the precise circumstances that induce in culture what is a normal programmed event in the life cycle is not yet known.

Physicists and physics have current problems which are much publicised. Beset by the multiplicity of their sub-atomic particles, they do not know the "glue" that holds the elementary particles together in the structure of matter. Even so the splendour of modern physical instrumentation enables enquiry to range to the limits of the cosmos and speculation to embrace the origin of the universe and the evolution of galaxies and of planets such as our own.

But modern biology also has its seemingly insoluble but challenging problems though these may be less dramatically publicised. When Darwin and the origin and evolution of life on earth are again being called into question one may rightfully ask the very pertinent question. How was matter first so organised that it could independently perform useful work? Also, having been so organized in cells (as in modern plants) how may they without loss of totipotency display the observed range of diversity in form and function? Genetics tells us that continuity of established traits is maintained in self-reproducing systems and, development shows that within tolerated limits diversity may be established; albeit the means by which genetically transmitted characteristics are regulated is still obscure. Genetics and molecular biology do not tell us how out of matter and energy life emerged. 'Modern' proposals to achieve this *de novo* seem to presuppose the prior existence of elaborated biological organisations that it is the ultimate purpose to explain. But has this essay a useful and final message?

10. Epilogue

New information may well continue to expand at its accelerating rate. Minutiae about every topic in plant physiology and botany may continue to overflow even the new journals as rapidly as they appear. Papers may get even shorter and focus only upon single and often trivial events. Readers who may increasingly scan only the tables of contents by title may not even pause to wonder where it all leads. The same furrows may be ploughed even narrower and deeper. But perhaps this celebration volume may induce some to see that it is not now enough to pursue "reductionism" vertically without relating it "horizontally" and "holistically" to see how the whole scheme of things, animate and inanimate, works. The final dilemma, that overrides all the details, is the ultimate mechanism of their control. Hopefully a general synthesis, as between botanists and physicists may emerge. Botanists comprehend and convey the respect for the organisation of plants, their cells and organelles. The physicists may be able to speculate upon how the 'breath of life' representing order and reduced entropy, was breathed into an inorganic world of matter and energy. If so, and in consort, they may

be able to comprehend the problems not only of the origin of life of evolution and inheritance but also of development and diversity.

But the formidable secrets of nature are not those that can be easily resolved in simplistic terms for, highly improbable and indirect means utilising complex organisations and structures have been elaborated to achieve what often seem to be simple objectives.

However detailed is the modern description of cells and organisms in terms of their material content, their chemistry, their physical structure, their morphology, their genetics they only operate in life in-as-much as it is all integrated into a coherent whole which, today, can be respected but not defined. In this sense biology rivals cosmology in its challenge.

The tremendous strides made by molecular biology and genetics in the understanding of unit controls over chemical events have not yet covered the obvious and long familiar situations in which nature achieves physical controls over large blocks of very diversified information. Morphological information that controls diversity in the plant body during the life cycle; biochemical information that controls the responsiveness of composition to form and environments during morphogenesis and physical information that ensures that during development and in changing environments the parts of the plant body comprise a coherent stable whole.

Plant cell physiology should not be complacent till these challenges are met.

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Photooxidative destruction of chloroplasts and its consequences for anthocyanin synthesis

H DRUMM-HERREL, R BERGFELD and H MOHR
Biological Institute II, University of Freiburg, D-7800 Freiburg i. Br., Germany (FRG)

Abstract. Electron microscopic and biochemical evidence is given that phytochrome-mediated formation of juvenile anthocyanin in the epidermis of the cotyledons and in the subepidermis of the hypocotyl of the mustard (*Sinapis alba* L.) seedling does not depend on intact plastids. Under experimental conditions where the plastid compartment is badly damaged and expression of plastid genes seems impossible anthocyanin formation proceeds normally. It is concluded that neither the plastids nor the vacuole are involved in the process of anthocyanin biosynthesis.

Keywords. Anthocyanin formation; destruction of plastids; phytochrome; *Sinapis alba*.

1. Introduction

Anthocyanins belong to a class of flavonoids which is ubiquitous in higher plants. Formation of anthocyanin in peripheral cell layers of seedlings ("juvenile anthocyanin") is considered a photoprotective means to attenuate the light absorbed by porphyrins, in particular by protochlorophyll and by chlorophyll during the early phase of greening where chlorophyll is still photolabile (Oelze *et al* 1983; Mohr and Drumm-Herrel 1983).

The present paper deals with the question of whether plastids play a role in anthocyanin synthesis. While it is agreed upon that anthocyanins accumulate in the central vacuole of the plant cell (Wagner 1979) the subcellular localization of the different steps of anthocyanin synthesis is still controversial. In particular, it has been claimed repeatedly (*e.g.* Saunders and McClure 1975; Weissenboeck *et al* 1976; Charriere-Ladreix *et al* 1981) that flavonoid biogenesis takes place within, or at least depends on (Podstolski 1981), the plastid organelle.

On the other hand, it was found that light-mediated anthocyanin synthesis in the hypocotyl of tomato (Drumm-Herrel 1984) and in the cotyledons of mustard (Reiß *et al* 1983) was not inhibited in the presence of herbicides such as Norflurazon or Difunon which inhibit carotenoid synthesis and thus cause photobleaching of chlorophyll and photodecomposition of plastids in strong white light.

In the present study anthocyanin synthesis in the mustard (*Sinapis alba* L.) seedling was investigated. Anthocyanin (five anthocyanins all of them containing cyanidin as the aglycone; Oelmüller and Mohr 1984) appears only in the epidermis (cotyledons) or in the subepidermis (hypocotyl) and its synthesis is induced by phytochrome (Oelmüller and Mohr 1984).

The following questions were asked: (i) What kind of plastids is contained in those cell layers (epidermis, subepidermis) which produce anthocyanin? (ii) Do these plastids respond to the herbicide treatment with photodecomposition in strong white light and what are the consequences for anthocyanin formation?

2. Material and methods

Mustard (*Sinapis alba* L.) seeds (harvest 1979) were sown and seedlings were grown under strictly standardized conditions at 25°C as described previously (Mohr 1966).

The herbicide Difunon, EMD-IT 5914, DF, 5-dimethylamino-methylene-2-oxo-4-phenyl-2·5-dihydrofuranecarbonitril-(3) has been applied as described previously (Frosch *et al* 1979). Difunon inhibits synthesis of carotenoids almost completely (by more than 99%) at the concentrations used ($2 \cdot 10^{-5}$ M) without affecting adversely morphogenesis of the seedlings (Reiß *et al* 1983).

For light treatment, seedlings were kept in continuous light from sowing onwards. Beside our standardized far-red (FR) light field (3·5 W m^{-2}; for technical data see Kochhar *et al* 1981) we used a strong white light field: 12 densely packed Osram fluorescent tubes (6L 20 W/25 and 6L 20 W/15 tubes) the emission of which was filtered through two glass filters (2 mm each) with 50% transmission at 330 nm to eliminate traces of UV below 300 nm. The light flux was 12000 lux (~ 30 W m^{-2}).

Anthocyanin contents were determined according to Oelmüller and Mohr (1984), carotenoids were assayed according to Frosch and Mohr (1980), and chlorophylls according to Ogawa and Shibata (1965).

Electron microscopy was performed as described by Frosch *et al* (1976). The

Table 1. Amounts of carotenoids and chlorophylls in mustard seedling cotyledons as affected by the herbicide Difunon, (2×10^{-5} M), applied at time of sowing. For light treatment, seedlings were kept in continuous light from sowing onwards

Treatment	Amount of carotenoids (n mol × pair of cotyledons^{-1})	Amount of chlorophylls (p mol × pair of cotyledons^{-1}) Chl-a*	Chl-b
48 hr WL	—	—	—
H_2O	787	2·570	0·590
DF	15	0·048	—
60 hr WL	—	—	—
H_2O	1903	9·190	2·280
DF	20	0·078	—
48 hr FR	—	—	—
H_2O	402	0·108	—
DF	18	0·134	—
60 hr FR	—	—	—
H_2O	931	0·352	—
DF	20	0·384	—
48 hr D	—	—	—
H_2O	196	0·058*	—
DF	16	0·060	—
60 hr D	—	—	—
H_2O	338	0·094	—
DF	18	0·112	—

(WL, white light; FR, far-red light; D, darkness; DF, Difunon.

*Chl-a after a saturating WL-pulse, representing the amount of photoconvertible protochlorophyll(ide).

herbicide which is not commercially available was kindly supplied by Professor P. Böger (Konstanz FRG).

3. Results

3.1 *Contents of carotenoids, chlorophylls and anthocyanin*

As expected (Reiß *et al* 1983) the application of Difunon ($2 \cdot 10^{-5}$ M) leads to an almost complete inhibition of carotenoid synthesis in the mustard cotyledons under all treatments (table 1). Correspondingly in white light (WL) (12000 lux) chlorophyll accumulation is prevented by Difunon whereas accumulation of chlorophyll in FR light and of protochlorophyll in darkness is slightly stimulated (table 1). Our standardized FR light was previously shown to be ineffective with regard to photooxidation of chlorophyll in the absence of carotenoids (Frosch *et al* 1979). Anthocyanin accumulation is slightly less in the presence of Difunon (table 2). This is due to a transient delay of synthesis in the presence of the herbicide (Reiß *et al* 1983; figure 8).

Table 2. Amounts of anthocyanin in cotyledons and hypocotyl of the mustard seedling as affected by the herbicide Difunon (DF, 2×10^{-5} M), applied at time of sowing. For light treatment, seedlings were kept in continuous light from sowing onwards.

	Amount of anthocyanin $[A_{535}]$*	
Treatment	Cotyledons	Hypocotyl
48 hr WL	—	—
H_2O	0·432	0·125
DF	0·385	0·101
60 hr WL	—	—
H_2O	1·237	0·191
DF	0·903	0·174
48 hr FR	—	—
H_2O	0·423	0·191
DF	0·402	0·184
60 hr FR	—	—
H_2O	1·082	0·310
DF	0·930	0·302
48 hr D	—	—
H_2O	0·004	0·002
DF	0·005	0·003
60 hr D	—	—
H_2O	0·018	0·004
DF	0·016	0·003

(WL, white light; FR, far-red light; D, darkness).
* A_{535} at time t was corrected for A_{535} at 24 hr after sowing. For justification of this correction see Oelmüller and Mohr (1984).

3.2 *Ultrastructure of plastids in the lower epidermis of mustard cotyledons*

The plastids of Difunon-treated material, 48 hr after sowing, exhibit hardly any internal structure in white light while in the herbicide-free controls (pro-)thylakoids can be detected. In FR light and in darkness no effect of Difunon treatment can be seen.

At 60 hr after sowing (figure 1) the ultrastructure of plastids from the lower epidermis of the cotyledons is not affected by the herbicide neither in continuous FR light nor in darkness. On the other hand in the presence of herbicide white light leads to a destruction of the internal structure of the plastid (WL/Difunon). The system of thylakoids has disappeared, except for a very few isolated thylakoids which seem to be attached to the envelope. Plastid ribosomes are no longer detectable, while cytosolic ribosomes are not affected. The plastid envelope does not show any signs of damage.

The same observations were made previously with the much larger plastids of the mesophyll cells of the mustard cotyledons (Reiß *et al* 1983).

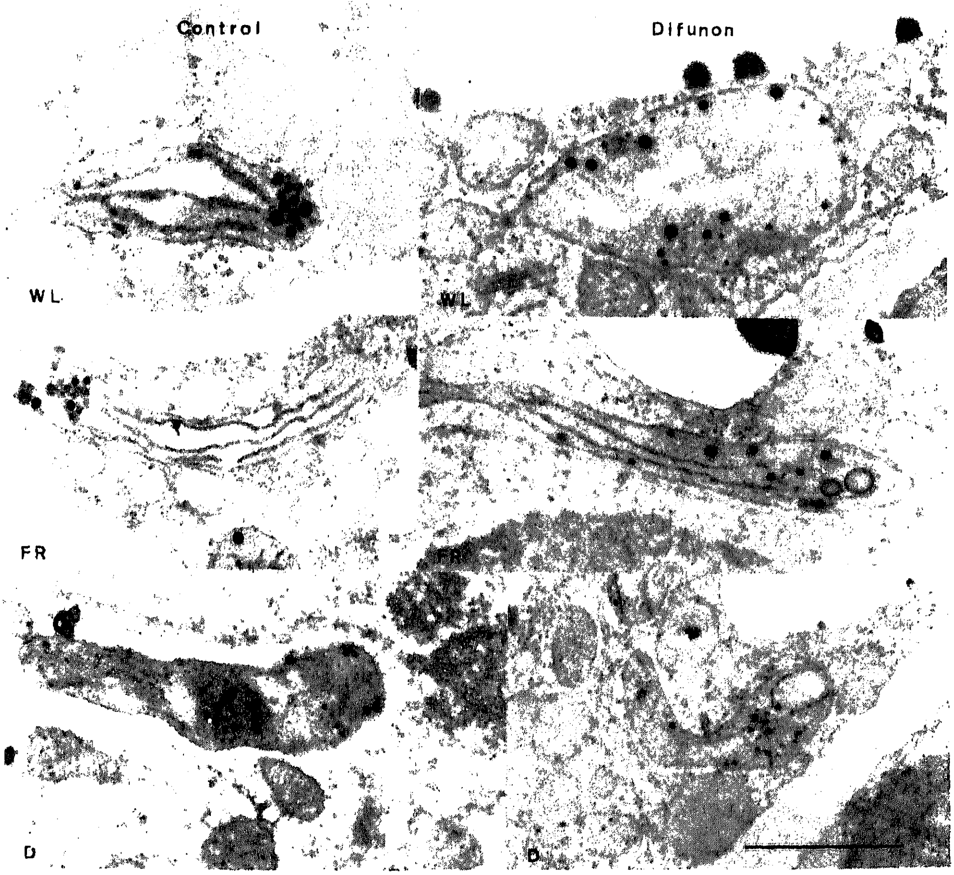

Figure 1. Sections through plastids from lower epidermis cells of mustard cotyledons. Difunon ($2 \cdot 10^{-5}$ M) was applied at the time of sowing. Seedlings were grown in the presence (Difunon) or absence (Control) of the herbicide for 60 hr under continuous light. (WL, white light; FR, far-red light; D, darkness. Scale bar = 1 μm).

3 *Ultrastructure of plastids in the subepidermis of the mustard hypocotyl*

1 the hypocotyl of mustard anthocyanin accumulates only in the subepidermis. This is common feature in many species of dicotyledonous seedlings (Nozzolillo 1972).

In WL and in the absence of Difunon the anthocyanin producing subepidermal cell yer contains normal chloroplasts with large starch grains (figure 2). In Difunon-eated seedlings the plastids remain small in while light and almost structureless. hylakoids and plastid ribosomes are almost lacking while the envelope seems to main intact.

Despite this severe damage there is no significant impairment of anthocyanin nthesis (table 2). In FR light and in darkness no effect of the Difunon treatment can be etected (see figure 2).

In epidermal cells of the hypocotyl (which do not accumulate anthocyanin) the lastids respond to Difunon in the same way as in the epidermis of the cotyledons hotographs not shown).

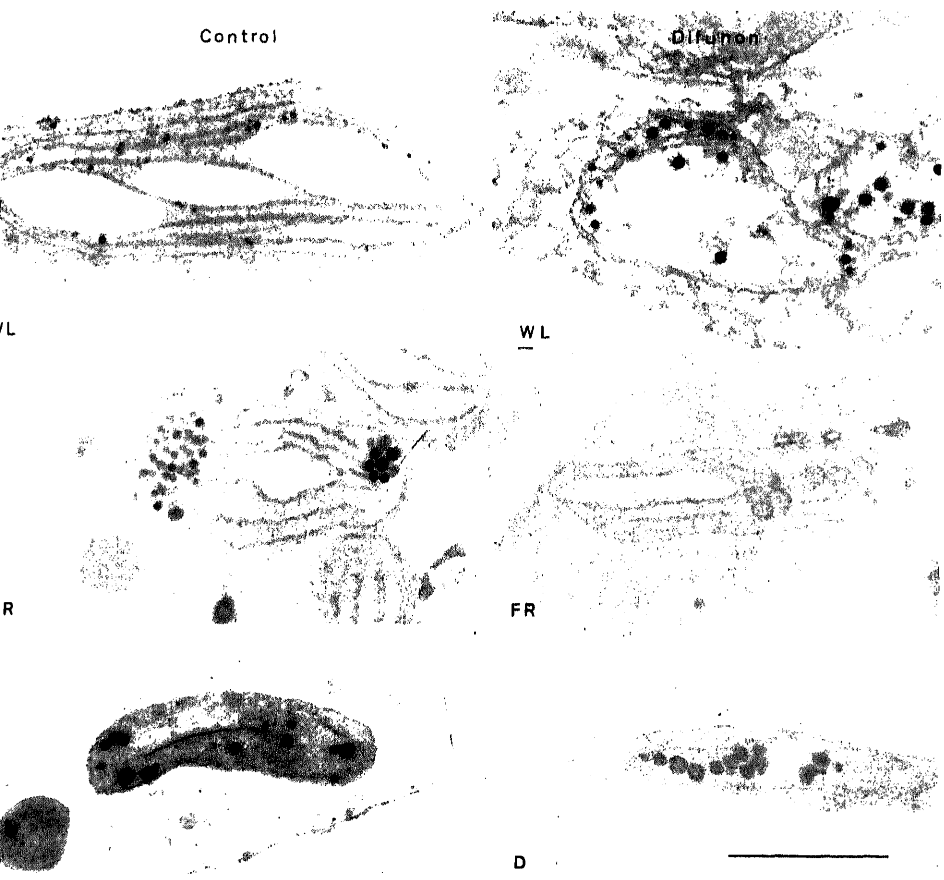

Figure 2. Sections through plastids from subepidermal cells of mustard hypocotyl. Difunon ($2 \cdot 10^{-5}$ M) was applied at the time of sowing. Seedlings were grown in the presence (Difunon) or absence (Control) of the herbicide for 60 hr under continuous light. (abbreviations and scale same as in figure 1).

4. Discussion

In WL (12000 lux) the cotyledons and the hypocotyl of Difunon-treated mustard seedlings do not contain normal chloroplasts but only small chlorophyll-free rudiments whose internal structure has almost disappeared. Plastidal gene expression is no longer detectable under these circumstances (Reiß *et al* 1983). Nevertheless, anthocyanin formation proceeds at the normal rate after a short transient delay (Reiß *et al* 1983).

Regarding the involvement of plastids in flavonoid biosynthesis (as postulated repeatedly by several laboratories, *e.g.* (Weissenboeck *et al* 1976; Knogge *et al* 1981; Nishizawa *et al* 1979; Saunders and McClure 1975; Charriere-Ladreix *et al* 1981), our data show that biogenesis of anthocyanin (cyanidin glycosides) in the mustard seedling does proceed at the normal rate even though the plastid compartment is badly damaged and expression of plastid genes seems impossible (Reiß *et al* 1983). Obviously formation of juvenile anthocyanin does not depend on intact, functional plastids.

In accordance with a previous study (Reiß *et al* 1983) we conclude that anthocyanin biosynthesis takes place exclusively in the cytoplasm. Neither the plastids nor the vacuole (Jonsson *et al* 1983) seem to be involved in the process of anthocyanin biosynthesis.

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hysiology of flower bud growth and opening

H Y MOHAN RAM and I V RAMANUJA RAO
Department of Botany, University of Delhi, Delhi 110007, India

Abstract. Flower growth and opening are commonplace events, but physiologically intricate and inadequately explained. In this review, we have brought together and evaluated information on this subject to focus attention on the dynamic facets of flower development. In particular, the physiological basis of flower bud dormancy, nature of cleistogamy, mechanism of flower bud growth and turgor maintenance and role of stamens in corolla growth have been examined. The regulation of flower movements and opening by temperature and light, and circadian rhythms in flower opening have been discussed, alongwith a consideration of the role of the petal epidermis in light perception.

It is emphasized that studies on flower physiology need to be intensified in view of the lacunae in our basic knowledge as well as to provide a sound basis for improving yields of both agricultural and horticultural crops.

Keywords. α-amylase; blossom showers; corolla; flower bud dormancy; flower bud growth; flower opening; flower movements; flower physiology; invertase; stamens; coffee; gladiolus; *Kalanchoe*; *Lamium*; *Pharbitis*; *Ruellia*; *Turnera*.

Introduction

ngiosperms constitute the dominant and most ubiquitous group of vascular plants ıd occupy a wide range of ecological habitats. The presence of a flower that bears vules concealed in a hollow ovary makes them unique. Defining what a flower is and ɔw it came to be established in the long course of evolutionary history have engaged e interest of generations of botanists. Lack of fossil evidence and the deep gaps ɛtween what is presently considered as the most primitive flower and the most highly volved anthostrobilus of fossil ancestors are serious handicaps in tracing the origin of ie flower. Several widely divergent viewpoints have been expressed in interpreting the ndaméntal nature of the flower, which has a varied structure and organization.

Although angiosperms have breeding systems other than sexual reproduction, the ain functions of the flower are: formation and release of microspores, reception of ɔllen, guidance of male gametophytes, gametic union, formation of seeds and ssociated dispersal structures and auxillary functions for the attraction or exclusion of ıimal vectors (Pijl 1978). Flowers display various adaptive mechanisms to speed up volutionary responses to meet the selective pressures by promoting genetic recombiıtion and gene migration.

On account of their economic, aesthetic and scientific value, flowers have captivated ıe attention of professional and amateur botanists alike. Naturalists have also been ·awn to the study of flowers because of the close interrelationship between the latter ıd mammals, birds and insects.

For a student of developmental botany the transition from the vegetative to the productive phase is a remarkable event. Despite the impressive amount of literature ı flower initiation, our present knowledge about flower development, involving ɔecific changes in gene function for the differentiation of floral organs in a close

succession on the floral axis is quite incomplete. The understanding of the inherent genetic mechanisms and their modification by the environmental and physiological factors are challenging questions for the plant scientist.

A glimpse into the work of earlier scientists such as Pfeffer (1897), Goldsmith and Hafenrichter (1932), Ball (1933, 1936) and Bünning (1929) on flowers displays originality of approach, a thoroughness in establishing facts and a penetration in understanding complex phenomena about flower structure, growth, movements and functions. The optical and scanning electron microscopic facilities, analytical tools, photographic appliances and the tissue culture technique available to the modern plant scientist are so refined that dynamic phenomena like flower growth and development can be studied not only in depth but also in a multidisciplinary perspective.

On account of limitation of space, only a few select aspects of flower growth are dealt with. Floral morphogenesis as understood through the application of *in vitro* culture (Johri and Ganapathy 1967; Mohan Ram and Jaiswal 1974; Konar and Kitchlue 1982) and experimental modification of flower sex have been covered elsewhere (Heslop-Harrison 1972; Mohan Ram 1980; Durand and Durand 1984).

As corolla generally constitutes the most conspicuous part of the flower, corolla expansion and divergence are considered synonymous with flower growth.

In the account that follows, material from classical works that have remained remarkably novel are drawn to illustrate poignant points and advances made in the recent years are discussed at appropriate places.

2. Genetic and environmental regulation of bud dormancy, corolla growth and opening

2.1 *Bud dormancy*

2.1a *Low temperature induction of growth*: In most plants flower buds, once initiated, follow a continuous course of development leading to anthesis and fruit formation. In some perennial plants especially the temperate deciduous trees, flower buds undergo a period of dormancy. Bud dormancy is broken by exposure to low temperatures. Under natural conditions chilling (1–10°C) in winter fulfils this requirement. If the winters are mild and do not extend over a sufficient length of time, the buds fail to blossom and set fruit. In sour cherry, bud growth is arrested after the sepal and petal primordia have been formed, anthers are at the stage of sporogenous cells and ovule primordia have not yet been differentiated (Felker *et al* 1983).

In sour cherry, flower bud chilling is accompanied by meiosis, first in the anthers and then in the ovule. Importantly, starch accumulation takes place with the progression of chilling. Ultrastructural studies indicate that during dormancy, carbohydrate metabolism plays a more crucial role than nuclear events (Felker *et al* 1983). However, in *Rhododendron* flower buds, starch accumulation occurs before the rest period and its utilization is postponed until the last weeks of cell expansion prior to anthesis (Schneider 1972).

Temperature is an important factor affecting flower initiation and development in bulbous plants (Hartsema 1961). Low temperature promotes the translocation of gibberellin-like substances from the bulb scales of iris to the shoot apex (Rodrigués-Pereira 1964). Whereas the concentration of gibberellins in the bulb scales does not vary

ch, a larger bulb would contain more total gibberellins than a smaller one. Work on cissus, tulip and hyacinth (see Rees 1972) has shown that flower initiation and ferentiation takes place in warm temperatures, but flower maturation and anthesis rapid only when the plants become exposed to low temperatures during or after wer differentiation. Although the precise role of gibberellins is not known, it has been ggested that in iris they are involved in flower initiation and flower stem development l in tulip in flower extension.

n narcissus flower primordia may be laid down one year before anthesis. Thus at the e of planting, a bulb contains already formed flower buds. In *Zephyranthes*, flower iation alternates with leaf formation throughout the growing period. Thus tiny nature buds and large buds approaching the point of anthesis occur in a dormant te in the bulb. In *Z. rosea* flower growth and opening are promoted when the nperature is lowered in the presence of water (Kerling 1949). It is also important that period of low temperature exposure exceeds 10 hr. Kerling (1949) was of the opinion t low temperature caused auxin release which promoted pedicel growth. Holdsworth 61) working with *Z. citrina*, *Z. tubispatha* and with two species of *Pancratium* showed t increased soil moisture after rains could be the factor triggering gregarious wering. The situation is thus reminiscent of that in coffee.

b *Role of moisture in breaking bud dormancy in coffee*: In certain coffee growing as, flower buds undergo a period of rest prior to meiosis after attaining a certain stage development. Bud opening occurs only after receiving a rainfall—appropriately med "blossom shower" (Went 1917; van der Meulen 1939; Mathew and Chokkanna 61). In Chickmagalur area in Karnataka, flower buds are initiated in October and nain dormant until April the following year. Failure of blossom showers can cause a ious set back in coffee production. Leliveld (1939) had noted that meiosis in *C. nephora* was dependent on the occurrence of rain. Mes (1957a) showed that flower l dormancy could be broken and meiosis triggered by submerging the bud-bearing nches of *C. arabica* in water or supplying water by other means. Water treatment of gs bearing buds has now become a standard method for studying meiosis in *Coffea* (Sreenivasan 1983).

According to Alvim (1958) the moisture effect is also dependent on temperature, her temperatures being more productive. Whereas Mes (1957b) believed that rmancy was caused by water stress in the young flower buds, Alvim (1960) observed t water stress was not the cause of dormancy but a condition necessary for ercoming it. It was only after experiencing a period of water stress that coffee plants come receptive to water, resulting in resumption of flower bud growth. Continuous wth under saturated conditions does not promote flower bud growth.

The dormant coffee flower buds are covered with a gum-like substance (Gopal *et al* 75). The coffee planters in India believe that this gum-like substance has some role in wering and that it has to first dissolve in the blossom showers before the buds can ume active growth. Chemical analysis of this gummy material has shown the sence of growth-promoting as well as inhibiting substances (Gopal *et al* 1975).

Browning (1973a, b) has proposed that the resumption of flower growth may depend the levels of endogenous abscisic acid, gibberellins and cytokinins in the flower bud. pal and Venkataramanan (1976) have observed that after sprinkler irrigation and ring the enlargement of the flower buds, there is a marked reduction in the total

content of growth-inhibiting substances with simultaneous increase in growth-promoting substances. Spraying of gibberellic acid (Alvim 1958) was effective in promoting the opening of flower buds. Alvim (1958) has suggested that the effect of moisture supply is through synthesis or activation of hormones (probably gibberellins).

2.2 *Cleistogamy*

In a majority of flowers the corolla opens to expose the stamens and the pistil (chasmogamy). There are some flowers in which the corolla remains permanently closed (cleistogamy). Production of both these floral forms is noticed in 287 species belonging to 56 families (Lord 1981). A marked reduction in the size of the corolla and stamens distinguishes the cleistogamous flowers.

Cleistogamy gives rise to offspring genetically very similar to the parents. However, the advantage of cleistogamy is that genes responsible for a local adaptive peak will be brought to a high localized frequency (Stebbins 1970; Williams 1975). Cleistogamy functions to slow or moderate evolutionary response (Thompson 1976). The role of chasmogamy is to speed up evolutionary responses to changing selection pressures by promoting both gene migration and reassortment (Thompson 1976).

Many species of *Viola* produce large quantities of seed by means of obligately cleistogamous (self-fertilized) flowers (Clausen *et al* 1964; Beattie 1978). Gopinathan and Babu (1984) have reported the occurrence of a population of *Vigna minima* the wild progenitor of rice bean—which bears obligate subterranean cleistogamous flowers.

A genetic basis for cleistogamy has been suggested for *Salvia cleistogama* (Burck 1906), *Commelina* spp (Uphof 1934), *Carthamus tinctorius* (Dille and Knowles 1975) and *Salpiglossis sinuata* (Lee *et al* 1976) although its expression is sensitive to environmental factors (Uphof 1938; Khoshoo *et al* 1969; Lee *et al* 1976).

In a study on *Ruellia* Khoshoo *et al* (1969) showed that during winter, the plants bear only cleistogamous flowers. The progeny of a cross between *R. tweediana* and *R. tuberosa* yielded a hybrid which was completely sterile and totally cleistogamous (Khoshoo *et al* 1969). Interestingly, gamma ray irradiation of the hybrid resulted in induction of chasmogamy (Raghuvanshi *et al* 1981). Hybrids formed between three tropical American species of *Ruellia* (*R. tuberosa*, *R. nudiflora* and *R. brittoniana*) were also completely cleistogamous but partly fertile (Long 1977).

Whether the closed corolla of cleistogamous flowers is a consequence or a cause of cleistogamy was debated by early workers. In *Lamium amplexicaule* Lindman (1908) observed that precocious maturation of anthers and early pollination could retard corolla expansion. Recently, Lee *et al* (1978) proposed that a burst of ethylene production in the cleistogamous flowers of *Salpiglossis sinuata* at pollination was responsible for arrested corolla expansion. However, Lord (1981, 1982) showed that the destiny of the two floral forms is determined much earlier, with the corolla shape divergence occurring subsequent to anther divergence.

Cleistogamous flowers produce a smaller amount of pollen and their germination invariably occurs within the anthers (Staedtler 1923). Lord (1982) has recently noted that in *L. amplexicaule*, cleistogamous and chasmogamous flower primordia are morphologically and histologically indistinguishable prior to meiosis. With the onset of meiosis and by the time the microspore mother cells are in prophase I, the two floral

es manifest their difference as evidenced by the reduced number of microspore ther cells in the cleistogamous anthers. Although clearly noticeable by the beginning meiosis, differentiation of the two forms must have occurred even earlier, since the crospore mother cells are reduced in number in the cleistogamous anthers. The cause this earlier differentiation is still unknown. Lord (1982) has indicated that this velopmental divergence in terms of cell division activity can now be narrowed down a short phase between the beginning of anther differentiation and prophase I of iosis. If lack of anthesis is excluded, the principal differences between the two floral ms is a slight quantitative modification in anther and corolla growth with calyx and noecium remaining unaltered. Lord (1981) believes that some mechanism that sociates development in the gynoecium/calyx with that of the androecium/corolla erates in cleistogamous flowers.

t is now possible to modify development and expression in cleistogamous/chasmo-nous flowers by hormone application. In cleistogamous *L. amplexicaule* (Lord 30), *Ruellia* hybrid (*R. tweediana* × *R. tuberosa*: Raghuvanshi *et al* 1981) and *llimia grandiflora* (Minter and Lord 1983), gibberellic acid (GA_3) application uces chasmogamous flowers, without altering anther morphology.

t is interesting to recall that water stress had been shown to increase cleistogamous wer production in *Stipa leucotricha* (Brown 1952) and *Bromus unioloides* (Langer l Wilson 1965). Recently, Minter and Lord (1983) have shown that both water stress l application of abscisic acid (ABA) induce cleistogamous flowers, causing a uction in both total flower number and chasmogamy. These authors have proposed t anthers are the target organ for hormonal control whose size is determined by the ative levels of ABA and GA_3 in the floral meristem. This contention disagrees with earlier observation (Lord 1980; Raghuvanshi *et al* 1981; Minter and Lord 1983) t anther morphology is unaltered.

The decrease in corolla size in cleistogamous flowers is attributed to reduction in her tissue (Lord 1979) since gibberellin application results in normal corolla ansion (Lord 1980; Raghuvanshi *et al* 1981). There is evidence to suggest that berellins produced in anthers are involved in corolla expansion (Murakami 1975; jan Bala 1982). It may be inferred that the gibberellin levels in the small anthers of istogamous flowers are too low to permit anthesis (Lord 1981). There is no erimental evidence to believe that anthesis is dependent on gibberellin levels. Once rmal corolla growth is attained through enhanced availability of gibberellins, hesis should follow as a normal consequence.

Among hydrophytes, cleistogamy has been recorded in several submerged and ating-leaved species (see Sculthorpe 1967 and references cited therein). In these the sed and submerged flower buds undergo self-pollination. *Ottelia ovalifolia* bears rmal aerial flowers in summer and small, submerged and cleistogamous flowers in ing or autumn or when the plants are overcrowded (Ernst-Schwarzenbach 1956). togamy occurs and the resulting fruits bear fewer seeds than the normal fruits. ether or not photoperiod regulates the formation of the two types of flowers in this nt is not clear. In some other submerged hydrophytes such as *Subularia aquatica*, *xa alternifolia* and *Ottelia alismoides*, self-pollination occurs within the closed wer buds, but the buds subsequently open (see Sculthorpe 1967). It was pointed out lier that in many land plants cleistogamy results from water stress. The occurrence cleistogamy in submerged water plants needs to be explained in physiological terms.

3. Energy requirement and turgor maintenance during flower bud growth

The magnitude of corolla growth varies with the type of flower and its ultimate size. In gladiolus, for example, the corolla adds nearly 16 times its fresh weight and over seven times its dry weight between the time the outer bract separates out until the corolla attains its full expansion (Rao 1979; Rajan Bala 1982). An entire mechanism has to be built up and sustained in the corolla to support the massive inflow of materials, since it is largely a non-photosynthetic organ. There are several reports which suggest that flowers are strong sinks for assimilates (Harris and Jeffcoat 1972). In gladiolus the inflorescence constitutes the main sink until anthesis (Ginzburg 1974; Robinson *et al* 1980; Shillo and Halevy 1981). Thus spikes harvested one week before the first floral bud opens out need exogenous supply of sugars for subsequent flower growth and opening (Rao and Mohan Ram 1981). Shillo and Halevy (1975) have shown that short days and reduced light intensity enhance flower abortion in gladiolus. Low light also impedes the development of inflorescence by increasing the competing ability of the corm for available assimilates.

Flower bud abortion or 'blasting' occurs as a result of competition for nutrients. For example in Easter lilies, Einert and Box (1967) showed that with increase in flower number per inflorescence, increased flower bud abortion took place. Smith and Langhans (1961) traced bud abortion to sudden moisture deficits. However, Mastalerz (1965) demonstrated that a reduction in the translocation of carbohydrate to the inflorescence caused by cooling the bud pedicel or bud removal promoted abortion. A direct dependence of flower bud abortion on the duration of darkness was also recorded. Working with lilies, Schenk and Boontyes (1970) noted that enclosure in the dark for one week caused bud blast and bud abscission in 27 and 72% of the cases respectively. In tulips and narcissus a rapid mobilization of reserves from the bulb to the shoot (amounting to three quarters of the bulb weight) occurs from sprouting of the bulb to flower opening (*see* Rees 1972).

Early flowering stages in several plants such as bougainvillea (Hackett and Sachs 1966), iris (Mae and Vonk 1974), tomato (Kinet 1977) and rose (Mor and Halevy 1980) are sensitive to light, and buds abort under low light conditions. Whether abortion of flower buds under low light intensities is caused by lower supply of photoassimilates or by a weakening of flowers as sinks because of hormonal or other factors leading to reduction in competing ability, has not been resolved satisfactorily. In carnations Harris and Scott (1969) showed that low light caused decreased rates of flower growth, but the rate of flower development was independent of the levels of assimilates available. These workers suggested that the processes controlling flower development regulate the partitioning of assimilates between the flower and the rest of the shoot system. Mae and Vonk (1974) concluded that bud abortion under low illumination cannot be attributed to lack of photosynthates. According to them hormone imbalance plays a key role in the assimilate distribution pattern and consequently in bud abortion, since application of hormones increases flowering. Kinet (1977) working with tomato also noted that light conditions were critical from the time of appearance (macroscopically visible) of the inflorescence onwards. He showed that the development of the inflorescence could be stimulated by direct application of benzylaminopurine and gibberellins under low light conditions. Hormone treatment favoured assimilate distribution to the inflorescence at the expense of young leaves. The work of Goldschmidt and Huberman (1974) has shown that wounding or application of

α-naphthaleneacetic acid to a single petal of citrus causes an increase in accumulation of ^{14}C-sucrose. Wounding of one petal causes accumulation in the remaining petals, often at the expense of the stamens.

Light has a promotory effect on the translocation of assimilates to reproductive sinks. For example in roses the terminal flower bud degenerates if it is enclosed in dark. Young rose shoots (2–3 cm long after flower initiation) are most sensitive to dark treatment (Mor and Halevy 1980; Mor *et al* 1980). It has been suggested that this occurs under conditions of low photosynthate availability, since the developing inflorescence is a weak sink that cannot compete effectively with vegetative sinks. It has been proposed that the rate of carbohydrate flow from source to sink is related to rates of activities of enzymes such as amylase and invertase (Hofmann *et al* 1963; Wünsch 1974; El-Fouly and Garas 1974). Fawzi and El-Fouly (1979) reported that following morphological initiation of floral primordia, amylase and invertase activities as well as total soluble sugar content increased in the shoot apex of carnation plants. In tomato also, the concentration of reducing sugars increased with floral development (Russell and Morris 1982). This was accompanied by an increase in acid invertase activity which rose to a maximum before anthesis. Flower bud abortion was correlated with reduction in the rate of dry matter accumulation, reducing sugars/sucrose ratios and in acid invertase levels. These workers suggested the operation of an invertase-mediated unloading mechanism for transported sucrose in the flower buds and noted that the transport of sucrose to the inflorescence might depend on the production of invertase rather than *vice-versa*. Limitations in invertase production under conditions of low photoassimilate availability would limit the import of carbohydrates and thus lead to flower bud abortion.

Ho and Nichols (1977) reported that the growing corolla in roses continues to import dry matter throughout its development and that reducing sugars (sucrose does not accumulate) and starch account for 50% of its dry weight. According to these workers, hydrolysis of stored starch is probably associated with flower opening in so far as decrease in water potential favours influx of water into the corolla tissue and promotes cell enlargement. In *Lilium corceum* the concentration of reducing sugars in the flower bud also increases until anthesis, accounting for over 30% of the dry weight of the corolla (Combes 1936). Reducing and non-reducing sugars account for 40–60% of the total dry weight of the ray florets in chrysanthemum (Pardha Saradhi, unpublished work). Sacalis and Durkin (1972) have also shown that sugars fed to cut roses and carnations accumulate largely in the petals, although probably only a small proportion is used metabolically. The function of sugar which is not directly involved in metabolism is unknown. Other than the obvious osmotic function in maintaining the turgidity of the petals, a part of the accumulated sugar in the petals is secreted as nectar. In gladiolus this occurs on anthesis and is also related to senescence at a later stage (Rao unpublished work).

In gladiolus α-amylase and acid invertase activities increase with the progression of corolla development and have a positive correlation with the amount of carbohydrates (Rajan Bala 1982). The decline in starch in late developmental stages can be correlated with the sharp rise in α-amylase activity and reducing sugar content. It may be inferred that the reducing sugars being osmotically active cause an influx of water, causing turgescence of expanded corolla (Winkenbach 1970; Rao and Mohan Ram 1980). Similar correlations between reducing sugar content, invertase activity and growth have been reported in the petals of *Ipomoea purpurea* (Winkenbach and Matile 1970)

carnations (Hawker *et al* 1976), rose (Ho and Nichols 1977) and in tomato inflorescence (Russell and Morris 1982).

In gladiolus, α-amylase is formed exclusively in the petal epidermis on perception of light. The enzyme is transported to the ground parenchyma where it hydrolyzes the extensive starch reserves (Rao and Mohan Ram 1980). In this respect it is comparable to the barley grain system. In barley, α-amylase synthesized in the aleurone layer causes the breakdown of starch stored in the endosperm. Whereas the barley grain represents a fully differentiated but quiescent system activated by gibberellin emanating from the embryo as a result of hydration, the gladiolus petal is a dynamic system triggered by light. The production of α-amylase is also regulated by the sequential basipetal differentiation of the epidermal cells (Rao 1982).

In gladiolus, the system of overlapping outer bracts which completely enclose the flower bud and their gradual separation represents a mechanism programmed for sequential exposure of successive buds to light and stimulation of α-amylase to ensure an orderly development of the buds. The outer bract has been shown to act as a natural qualitative light filter and regulates the production of α-amylase and petal growth by a red far-red control (Rao 1982; Rao and Mohan Ram unpublished work). A critical stage in flower bud growth in the spike of *Gladiolus* which is initiated by gibberellic acid and sustained by sucrose has also been identified (Rao and Mohan Ram 1982). This corresponds to the stage at which separation of the outer bract occurs, leading to light induction of α-amylase (Rao and Mohan Ram 1980). Buds not induced by light were shown to respond more significantly to GA_3 and sucrose than those induced by light. Since the separation of the outer bract results in light-induced α-amylase production and starch hydrolysis leading to petal growth, it has been proposed that growth promotion by GA_3 is related to light-induced petal growth at this specific stage (Rao and Mohan Ram 1982). The demonstration in gladiolus (Rao 1982) that the growth of the petals at different developmental stages is a function of the levels of α-amylase in those stages, strengthens the conclusion that the sugars released through α-amylase activity are directly involved in petal growth. Light-mediated α-amylase production could thus be an important step in the formation of an active sink to draw materials from the rest of the plant. One important role of continued and sequential basipetal starch hydrolysis in the gladiolus petal (Rao and Mohan Ram 1980) could be to maintain a constant osmotic as well as sink potential in the growing area of the petal, in spite of water uptake.

Investigations on cell and organ expansion have been largely confined to vegetative parts. Information on cell expansion in floral organs is scanty. This may be traced to lack of good experimental systems rather than to absence of interest. In fact, production of larger flowers has been an important concern of flower growers, which would require the understanding of the basic mechanisms involved.

An elegant method has been developed in this laboratory which involves the floating of ray florets (9 to 9·5 mm) of *Chrysanthemum morifolium* var 'Jyotsna', removed from the outermost whorl of young capitula in petri plates containing 30 ml of the test solution (Pardha Saradhi and Mohan Ram 1982). Using this technique it has been shown that KCl causes up to 33 % increase in elongation. The values for GA_3 and sucrose when used individually are 39·8 and 28·9 % respectively. Maximal growth response (82·8 %) is recorded in KCl + GA_3 + sucrose. It is inferred that the increased turgor resulting from sucrose-promoted potassium uptake along with GA_3-caused tissue extensibility accounts for the enhanced floret growth.

N,N'-dicyclohexyl carbodiimide (DCCD), a potent inhibitor of membrane-bound ATPase strongly inhibits the growth of ray florets of *Chrysanthemum* even under *in vivo* onditions, showing the importance of membrane-bound ATPases in ray floret xpansion (Pardha Saradhi, unpublished work). Ray floret expansion is also retarded y (2-chloroethyl) trimethyl ammonium chloride (CCC), an inhibitor of gibberellin iosynthesis (Pardha Saradhi unpublished work) implying that endogenous gib- erellins are involved in ray floret growth. The CCC effect can be overcome by imultaneous application of GA_3.

. Role of other floral organs in corolla growth

n most investigations on flower growth, emphasis has been on the growth of corolla as t generally constitutes the most conspicuous part of a flower and since the changes ccurring in it are dramatic. By and large, the role played by other floral organs in bud pening has been overlooked. For example, until recently, bracts were considered as rgans meant for the protection of the immature floral buds. The discovery of the rucial role of the outer bract as a light filter in the triggering of corolla growth in ladiolus (Rao and Mohan Ram 1980) demonstrated the close interaction between he different components of the flower bud. Historically, however, the stamens were he first floral organs shown to influence corolla growth (Lang 1961).

Developmentally there is a correlation among the various floral organs. Factors hich favour the development of the ovary also favour the sepals and those promoting tamen development also stimulate corolla expansion. In *Glechoma hederacea*, the ermaphrodite flowers have larger corollas than the female flowers (Plack 1957). xposure to long days or application of α-naphthaleneacetic acid to *Silene pendula* lants caused male sterility and reduction in corolla growth, but promoted ovary evelopment markedly (Heslop-Harrison and Heslop-Harrison 1958). In *Bryophyllum* nd *Kalanchoe* low light intensity causes abortion of the stamens and reduction in orolla development while not affecting ovary growth (Resende 1949/1951). Whereas ery little is known about the relationship of the sepals and the ovary, it can be oncluded that the stamens have a strong influence on corolla growth. The exact nature f this influence has not been defined so far. Thus the influence of an important event ke meiosis on corolla development has still not been understood.

Observations on natural anther abortion and male sterility as well as from xperimental emasculation indicate that functional anthers are necessary for the ormal growth and development of many flowers (Lang 1961). Male sterility and educed petal growth are sometimes correlated (Michaelis 1954). Application of male ametocidal compounds such as mendok and dalapon cause not only abortion of the porogenous tissue in the anthers but also result in poor growth and opening of the orolla in *Linum* (Rustagi and Mohan Ram 1971). Flowers of treated plants become rogressively smaller and cleistogamy increases with increasing concentrations of the ompounds. Kinoshita (1971) is of the opinion that a good deal of similarity exists in e mode of pollen abortion in chemically induced and genetically controlled male terile plants. The tapetal cells generally become hypertrophied or radially elongated, cclude the anther locule and crush the microspores.

In *Tulipa gesneriana*, spontaneous atrophy of stamens leads to a marked decline in obilization of starch into the bud and is followed by the decay of the entire bud

(Marré 1946). Interestingly in many Euphorbiaceae, the male flowers have petals whereas the female flowers are naked. It is important to recall a few exceptional cases such as banana and papaya in which the functionally female flowers bear larger perianth than the male flowers. Baker (1957) first pointed out the difference in size of pistillate and hermaphrodite flowers in gynodioecious and gynomonoecious plants. In *Glechoma hederacea* the female flowers on predominantly hermaphrodite plants and those on purely female plants are smaller than the hermaphrodite flowers (Plack 1957). She found that occasional flowers which have one, two and three aborted anthers are intermediate in size between the female and complete hermaphrodite flowers. Emasculation of the hermaphrodite flowers in bud condition reduces the size of the corollas in proportion of the number of anthers removed, with the corolla of the flower in which all four anthers had been removed being similar in size to the normal female flower. It was proposed that a hormone was released post-meiotically from the anthers since the critical stage for treatment in *Glechoma hederacea* was a 7 mm bud whereas meiosis is completed when the bud is 1 mm long. Wittwer (1943) had also found that the production of 'growth hormone' in staminate flowers occurs only after the onset of meiosis. According to Marré (1946) the effect of anthers on the growth of other parts of the flower ceases when the anther reaches maturity and the tapetum is used up. The maximal auxin production by the ovary of *Papaver* occurs when there is meiosis in the megaspore mother cells and mitosis in the surrounding tissues (Katunskij 1936).

In *Soldanella minima* the development of flower buds grown at 15°C or above became arrested at the stage of microspore mother cells in anthers (Lona 1968). This could be overcome by low temperature which not only promoted microsporogenesis but also subsequent bud growth and opening. Thus, microsporogenesis in this plant must be considered as the phase initiated only at low temperature. Application of gibberellic acid could substitute for the low temperature requirement of flower buds. Lona (1965) detected gibberellin-like activity in methanolic extracts of *Corylus avellana* pollen and that of other plants after cold treatment. However, the efficiency of GA_3 in promoting flower bud growth till full opening in plants where such 'intra-reproductive vernalization' is necessary is not always equal to that obtained in *Soldanella*, *Saxifraga*, etc. Thus, the development of *Draba azoides* flower buds till anthesis can be induced by gibberellin applications only with great difficulty. Some other plants do not even respond. According to Lona (1965) it is possible that other compounds may also be responsible for the "vernalization process" in these plants and that flower development may be controlled by a sequential complex of morphogenetic substances such that GA alone cannot mimic the cold effect.

The promotion of corolla growth by gibberellin was first demonstrated by Plack (1958) in *Glechoma hederacea*. Application of GA_3 to emasculated flowers was able to replace the stamen effect. She proposed that anther-derived gibberellin may probably be important in normal maturation of some flowers. Indoleacetic acid (Plack 1957, 1958), α-naphthaleneacetic acid, triiodobenzoic acid and L-proline did not have a similar effect. Lindstrom and Wittwer (1957) also observed an increase in petal size of *Pelargonium hortorum* treated with GA_3. Blake (1966) reported that gibberellins are involved in the growth of *Viscaria candida* flowers. Staby *et al* (1972) showed that 90% of terpene-synthesizing potential of cell-free extract leading to gibberellin synthesis of young tulip buds was derived from stamens. Murakami (1973) also reported that removal of anthers from flower buds reduced the final length of the floral tube in

Pharbitis nil. Murakami (1975) further demonstrated that anthers were a source of gibberellins in *Mirabilis jalapa.* The acetone-extractable gibberellin-like compounds in petals and stamens of *Pharbitis* increased rapidly after the petals emerged from the calyx, reaching a maximum 12 hr before anthesis and then declining markedly (Murakami 1973). The gibberellin content in stamens was relatively large in these plants and accounted for changes in gibberellins during corolla development. In *Impatiens sultani*, gibberellin and cytokinin levels increased during the transformation of a bud into a flower and later decreased after stamen abscission (Baltepe 1983).

In *Gladiolus* intact stamens promote the growth of isolated corollas. The increasing independence of the older stages without stamens in this plant suggests that the influence of stamens decreases as corolla growth advances although it does not stop. The influence of stamens on corolla growth appears to be through the production of gibberellins since the critical effect of stamens can be completely replaced by GA_3 or GA_3 + sucrose (Rajan Bala 1982). Recently Goldschmidt (1980) demonstrated that the ABA content of citrus petals which is initially low, increases gradually with a concurrent decrease in stamens as the bud development proceeded towards anthesis.

Ethylene is detrimental for flower growth in bulbous plants. The stage of flower development in tulips that is susceptible to ethylene is at pollen tetrad formation, which is attained by about four weeks after completion of flower differentiation. Perianth growth becomes stunted resulting in the production of 'open' flowers in which the anthers are exposed (*see* Rees 1972). The smaller flowers are also short-stemmed than normal flowers. Ethylene has been shown to cause anther necrosis after completion of flower differentiation with the perianth frequently remaining green at anthesis. Alternatively the entire flower dries up ('blasting'). Even endogenous ethylene production by the anthers of healthy flower buds during meiosis (especially at higher temperatures), leads to flower death (*see* Rees 1972).

5. Regulation of flower movements and opening

As flower opening is an essential requirement for cross pollination, plants have adopted various strategies to attain it at the most appropriate period in a given microenvironment. Although it has been recognized since long that flower opening is basically a result of greater expansion of the inner surface of the petals more than the outer, the means by which this is accomplished is diverse. Light and temperature are the most important factors that regulate flower opening, although it is to be expected that many factors may be operating simultaneously or successively. It is important to note that rapid turgor movements so commonly observed in the leaf movements have not been recorded in flower movements and opening. However, Pontecorvo (personal communication 1982) has observed that in some high altitude flowers growing in the Alps, closure of petals occurs almost within a few minutes when the sky above the flowers becomes covered by a passing cloud, causing a reduction in light intensity. As the clouds move away the flowers resume their open position. Light-sensitive turgor changes must account for such rapid flower movements and this aspect needs further study. It has also been observed in the capitula of certain Compositae such as *Helichrysum* and *Acroclinum* that opening is caused by desiccation and closing by treatment with water. Although this is dramatically noticed in the dried capitula, clearcut movements can be observed in the intact capitula also.

5.1 *Sun-tracking flowers and flowers that bend up and down*

Several flowers follow the sun's movement from east to west. In the sunflower, Shibaoka and Yamaki (1959) showed that the east-west movement was a growth phenomenon of the stem. The movement is probably regulated by the auxin content of the sides of the stem which face or are away from the sun. Placing the plants in continuous darkness does not terminate the east-west movements for several days, thereby showing that a circadian rhythm is involved. Whereas the precise mechanism has not been elucidated so far, in the leaves of the sun-tracking *Lavatera cretica*, a differential starch deposition in the lamina is thought to act as a "memory" for the leaves (Fisher and Fisher 1983). Asymmetrical deposits of starch are produced immediately after sunrise each day and these are believed to be stored until just before the next sunrise. The resulting solute asymmetries in the petiole are believed to produce anticipatory movements just before dawn.

The up and down movements of flowers have been shown to be a function of the differential growth of the pedicel. In *Kalanchoe blossfeldiana* (Bünsow 1953), the movement of the buds occurs daily till they are open. Transferring of the plant to continuous dark does not terminate movement for several days. Since this is a growth phenomenon of the pedicel, the regulation of flower movement could be dependent on the auxin-auxin inhibitor levels in the pedicel, as shown by Kaldewey (1957) in *Fritillaria*.

5.2 *Effect of temperature on flower opening*

Investigations on the effect of temperature on flower opening were initiated during the second half of the 19th century. In *Crocus* and certain varieties of tulip, flower movement was shown to be dependent on a small change in temperature and was nearly independent of light. Andrews (1929) found that in *Crocus* increase of only 0·2C was sufficient to initiate the opening movement. For tulips the temperature increase necessary was 1 C (Andrews 1929). A fall in temperature (which is about equal to that necessary for initiating opening) brought about flower closure. Böhner (1934) mentions a critical temperature, below which tulip flowers have a tendency to close and above which they open.

It is generally agreed that the opening and closing of flowers are not caused by turgor changes but are brought about by differential growth of the two sides of the tepals (Pfeffer 1897; Bünning 1929). It was earlier believed that gentle warming of the inner surface of the tepal caused rapid growth, whereas the outer surface did not respond. Bünning (1929) demonstrated that warming the outer surface of the tepal while keeping the inner one cool caused growth on the outerside. The closing results from a slower growth response of the inner side at lower temperatures in comparison to the outerside. Crombie (1962) has written a critical review on thermonasty in flowers.

By removing one or both the epidermal layers of petals Märkert (1931) showed that the growth reaction (opening or closing) occurred chiefly in the mesophyll region. Böhner (1933, 1934) concluded that whereas the growth of the mosophyll cells was the principal mechanism, a controlling effect was exerted by the epidermal layers. There was little change in osmotic pressure and a permanent increase in length was obtained after every growth reaction (Bünning 1929; Böhner 1934). Wood (1953) concluded that the two surfaces of a tepal have different optima for growth. She found that the

nesophyll cells near the outer surface of the tepals of tulip had a temperature optimum or growth which was about 10C lower than that of the cells near the inner surface. This vas true even when strips of mesophyll and epidermis were taken from the inner and outer sides. When the temperature was raised from 7C to 17C, there was a marked ncrease in growth of the inner strip whereas the outer strip did not show much esponse. Lowering the temperature, however, caused the outer strip to grow at an ncreased rate while the inner strip showed little change. The reactions of isolated pidermal layers, although similar, were of a smaller magnitude than those of the orresponding mesophyll cells. A much greater growth response was found in complete epals. Carbon dioxide was shown to have a marked effect on the temperature optimum or growth, especially in the inner surface cells, with a rise in CO_2 concentration shifting he optimum to a lower temperature.

Studies on *Turnera elegans* (Ball 1936), *Cestrum nocturnum* (Overland 1960) and *Sida hombifolia* (Ugborogho 1980) have indicated that lower temperatures delay and high emperatures hasten flower opening. In contrast to this general situation, Kaihara and Takimoto (1979) found that lower temperatures at dawn promoted earlier opening in *Pharbitis nil* and higher temperature delayed it. These authors showed that the emperature to which the buds were subjected during the light period modified the ength of the dark period needed to promote rapid flower opening and that lower emperatures eliminated the suppressive effect of light on flower opening, such that at 20C the buds could open rapidly even in continuous light (Kaihara and Takimoto 1981a). It was also found that irrespective of when the low temperature treatment was begun, under continuous light all the buds flowered at about the same time. Kaihara and Takimoto (1981b) also determined that petals are sites of photo- and thermo-perception and that the curvature of the midribs is the primary force in flower opening.

.3 *Effect of light on flower opening*

.3a *Day-blooming flowers*: One of the earliest and detailed inquiries into the mechanism of flower opening was on *Turnera ulmifolia* L. var. *elegans* Urb. by Ball (1933, 1936). In this plant the petals of a mature bud emerge from the calyx in the evening and elongate rapidly during the night. Anthesis begins 1 hr after dawn and the flower is fully open in about another hour. The flowers close and wither within 3–4 hours. The closing of the flower is due to active hyponasty and is independent of withering. Light interruption of the dark period inhibits flower opening. Illumination during the second half of the night has a greater effect in inhibiting anthesis than that during the first half (Ball 1933).

Buds which were one day younger and scheduled to open the second morning after receiving one night's illumination were affected such that even though the corollas in these buds were contained within the sepals during the night when illumination was given, the flowers failed to open fully and the petals appeared crumpled at the tip (Ball 1933). He emphasized that in all cases the corolla expanded to its normal size but only its divergence did not take place. However, in *Hedera helix* Sigmond (1929) observed that flower buds illuminated during the night failed to open the following morning.

Ball (1933) showed that the petals and sepals of *Turnera* buds contain abundant starch which largely disappears by anthesis. He attributed the rapid expansion of the petals to increased osmotic pressure resulting from starch hydrolysis and elevated levels of

reducing sugars. In both petals and sepals, the hydrolysis of starch commenced at the tips and proceeded downwards. Exposure to light at night, which prevents buds from opening, impeded starch hydrolysis and the petals still contained large amounts of starch throughout the mesophyll. Ball (1933) emphasized that starch hydrolysis was more related to anthesis than to corolla growth. He stated "there may well be a causal relationship between the inhibition of starch and the absence of the epinastic expansion of petals" (Ball 1969).

The inhibitory effect of illumination on flower opening (also starch breakdown) was observed in several other plants (Ball 1933). Exceptionally, however, in *Cassia occidentalis* flower opening was inhibited by nocturnal illumination without affecting starch hydrolysis. Ball (1933) realized that mere hydrolysis of the existing starch reserves would be too insufficient to account for normal growth and opening of the flowers and that sugar movement from the rest of the plant was necessary. During the period of rapid expansion of the petals quick increments in fresh and dry weight were noted. Starvation of the shoots markedly reduced flower size. Continued starvation caused incomplete opening of the flowers. Schmucker (1928) suggested that carbohydrate changes might be responsible for the opening of flowers of *Cereus grandiflorus* and that the utilization of sugars during anthesis was indicated by a slight rise in flower temperature. Subsequently Ball (1936) observed that red light (650–700 nm) was as effective as white light in preventing flowers from opening, while blue light was ineffective. Red light inhibition was also correlated with lack of starch hydrolysis. Ott (1958) also recognized the importance of red light in causing flowers of *Ipomoea purpurea* to wilt without opening.

Flower buds of *Pharbitis nil* ordinarily open at dawn. When the photoperiod during the previous day is 10 hr or longer, the flower buds open after 10 hr of the onset of darkness. But if the photoperiod is 9 hr or shorter, the buds open about 20 hr after the onset of the photoperiod (Kaihara and Takimoto 1979). Exposure to fluorescent light (daylight) in the first 4 hr of dark period delays opening, but when given later, it has little effect. A 5 min pulse of red light given every 30 min delays opening. Far-red light reverses the action of red light. These observations suggest that the absence of Pfr during the first 4 hr of darkness is necessary for opening (Kaihara and Takimoto 1980).

5.3b *Night blooming flowers*: The last change from night to day appears to be the trigger for flower opening in night-blooming flowers and continuous dark inhibits opening in these flowers. Schmucker (1928) and Rauh and Zehender (1958) working with *Cereus*, and Arnold (1959) studying two species of *Oenothera* showed that the timing mechanism was probably located in the buds, as each bud must be exposed to the dark to light transition before it opens. Schmucker (1928) observed that exposure of plants to light at night and keeping them in dark during daytime caused the normal periodicity to become weaker gradually until it was replaced by a new one in which the plants opened in the morning. Stomps (1930) working with a hybrid *Oenothera*, found that plants kept continuously in dark retained the original periodicity for 6–8 days. In *Cestrum nocturnum* (night queen) also opening and closing in a cyclic manner could be maintained in constant light or darkness. Emission of the strong scent at blooming followed a circadian rhythm (Overland 1960).

5.3c *Physiological basis of flower opening*: Ordinarily flower buds of *Pharbitis nil* cut at early developmental stages open slowly and at varying times. When ABA is given to

he cut buds, opening is rapid and occurs even in continuous light at 25 C (Kaihara and 'akimoto 1983). These workers believe that ABA promotes flower opening by resetting he timing mechanism in a manner simulating dark treatment.

Working with *Kalanchoe*, Schrempf (1980) proposed that ABA does not directly ffect the circadian oscillator which controls opening time but rather influences the lriven oscillator (petal movement). Thus ABA dampens the amplitude of the petal novement rhythm but does not affect the period length of the circadian rhythm. Removal of ABA (10^{-6}M) and giving a light pulse increases the amplitude. Use of a igh concentration of ABA (10^{-5}M) results in permanent dampening of petal novement. He suggested that since ABA was taken up in the transpirational stream, the esults could be explained in terms of a rhythmic supply of ABA due to rhythmic ranspiration (zur Lippe 1957; Schrempf 1980). ABA is supposed to act on ion fluxes van Steveninck 1976) which correlate strongly with turgor changes leading to petal novement (Schrempf 1980). Interestingly, Goldschmidt (1980) reported that the level of ABA in the citrus flower increases in the course of development. (Earlier, Goldschmidt 1968 had indicated that the bending of citrus petals might be an auxin-nediated process).

The circadian rhythm in petal movement of *Kalanchoe blossfeldiana* is the best tudied flower system (Bünsow 1953). In this plant, the phase-response curve to high emperature pulses resembled the light response curve (Engelmann *et al* 1974a) and losely approximated those generated by a feedback mathematical model for the ircadian oscillator. The presence of lithium ions and deuteration were shown to slow he circadian clock in *Kalanchoe* petals (Engelmann 1972; Engelmann *et al* 1974b). In ontrast to the proposal of Schrempf (1980) that rhythmic water uptake was the cause or circadian sensitivity in regulation of petal movement of *Kalanchoe*, Eckhardt and Engelmann (1984) have recently shown that rhythmic uptake of vanadate (an ATPase ctivity inhibitor) is not sufficient to explain its time-dependent phase shifting effect. The dose-effect-curve for vanadate given at a phase when minimal delays are brought bout should show larger phase delays at higher concentrations, but the peak is at the ame concentration as at a phase where maximal phase delays are induced. Through he use of vanadate, Eckhardt and Engelmann (1984) have demonstrated that the lasmalemma ATPase plays a role in the functioning of the circadian rhythm of *Kalanchoe*.

.4 *The function of the epidermis in light perception*

t would be pertinent at this stage to consider the function of the epidermis in light erception since it would be the first petal layer to intercept light. The inhibition of pening by light documented above must occur consequent to light perception by the pidermis followed by transduction of the stimulus through chemical or electrical neans to the physically responsive part of the petal.

A crucial structural and biochemical role of the epidermis in light perception leading o petal growth has been demonstrated in gladiolus. Rao (1979, 1982) has shown that he epidermal cells of petals of gladiolus possess microlenses. These are formed by the utward growth of the outer radial wall of the epidermal cells resulting in the formation of curved structures which are thicker in the middle than at the edges. These nicrolenses act to focus light specifically onto the nucleus which is situated in the entral zone of the inner radial wall of the epidermal cells. Prior to the development of his focussing microlens, the nucleus is randomly situated in the epidermal cell and later

moves over to occupy the focal point. The development of the microlens is characteristic of differentiated cells; undifferentiated cells have an ordinary flat surface. Importantly, it is only after full differentiation as characterised by formation of the microlens that the epidermal cells become competent to be induced by light (Rao 1982). These then start producing α-amylase which is later transported to the ground parenchyma where hydrolysis of the accumulated starch takes place (Rao and Mohan Ram 1980).

The shape of the epidermal cells of the petals is useful in the absorption and re-radiation of specific bands of the spectrum (Kay *et al* 1981). This becomes crucial as the final colouration of the flower is critical in terms of insect vision in order to provide adequate guidance for their approach and alighting, leading to pollination. Petal pigments such as anthocyanins and flavonoids are contained in the vacuoles of the epidermal cells and light is reflected back from an aerenchymatous, unpigmented mesophyll. In certain *Ranunculus* and *Anemone* species light is reflected from a specialized layer of starch grains (Kay *et al* 1981).

Haberlandt, the celebrated physiological plant anatomist whose works and ideas were much ahead of his time (1914) had observed that the outer wall of epidermal cells frequently protrudes out in a slightly papillose manner and that in many shade plants, this tendency is accentuated such that large conical epidermal papillae are formed. He initially believed that the epidermal papillae might act as condensing lenses to concentrate the available light upon the chloroplasts of the palisade cells, presumably to increase photosynthesis. Later, he conceptualized that "lens-action" of epidermal papillae is primarily connected with the perception of photic stimuli. Stahl (*see* Haberlandt 1914) advocated that the conical epidermal papillae of velvety leaves enabled them to absorb oblique rays of light which would have been totally lost if the outer epidermal cells were of the ordinary flat type.

The cave moss, *Schistostega* has peculiar epidermal cells in which the upper cytoplasm acts as a refractive lens to focus light on specially-oriented chloroplasts (Richards 1932). Lee and Lowry (1975) observed that the epidermal cells of *Selaginella wildenovii* which are "egg-shaped" with a convex outer wall and having chloroplasts in a "peculiar position" distal to the leaf surface, might also function as lenses similar to that in *Schistostega*. These workers further reported that the epidermal cell wall surfaces functioned as thin-interference filters causing an increased reflection of photosynthetically less active light (400–500 nm) with increased penetration of the most photosynthetically active range (600–650 nm). This is an important adaptation because light reaching the floor of rain forests in which these plants occur is extremely limiting and is deficient in photosynthetically active wavelengths.

There is a growing recent interest in studying the role of epidermis in flowers. The full impact of the function of this bounding layer of the plant is yet to be fully realized.

6. Conclusions

The process of flower growth and opening appears simple and commonplace. Yet physiologically it is intricate, involving an interplay of genetic and environmental factors. In adapting to a wide range of bioclimatic conditions, plants have displayed several mechanisms to accomplish flower opening. Plants have also evolved strategies

produce flowers best suited to the fluctuating seasonal conditions. Cleistogamy is a oduct of such an effort.

The usefulness of studies on flower growth and opening discussed in this paper have en grossly underestimated. Staple food is all seed and seeds are borne by successful wers. It is, therefore, critical that a flower bud develops a strong sink capability. The ndamental studies discussed by us have demonstrated that light, stamens, petal idermis, α-amylase and invertase form important components in the development of ch a capability. In floriculture, the application of scientific principles and knowledge ve yielded unprecedented dividends. Flower buds of some floral crops can now be ndled, cold-stored and marketed like any other agricultural commodity. Left to the garies of nature, inadequate overcoming of flower bud dormancy could mean the ilure of an entire fruit crop. Can there be a greater attraction for work in the area of wer physiology?

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Photoacoustic characterisation of the *in vivo* levels of chlorophyll a in the adaxial and abaxial sides of the leaf

A S KOLASKAR, K R NAIDU, Y SEETHAMBARAM and V S RAMA DAS*

School of Life Sciences, University of Hyderabad, Hyderabad 500 134, India

*Department of Botany, Sri Venkateswara University, Tirupati 517 502, India

Abstract. Photoacoustic spectroscopy was applied to determine the distribution of chlorophyll a in the abaxial and adaxial sides of the leaf in 27 species of angiospermous plants. Two distinct patterns were observed in the ratio of the level of chlorophyll a in the abaxial to adaxial side of the leaf in the monocotyledons and in dicotyledons plants. The ratio was not correlated with the C_3 or C_4 type of photosynthetic pathways.

Keywords. Photoacoustic spectroscopy; chlorophyll a; Abaxial-adaxial ratio.

1. Introduction

The pigment chlorophyll a plays a crucial role in the photosynthetic process. It is also well known that nature provides a supra abundance of chlorophyll for photosynthesis. Though in the past the content of chlorophyll was widely estimated *in vitro* comparatively few studies were made of *in vivo* distribution (French 1960). Particularly the distribution of chlorophyll a on the two sides of a leaf in various plants was hitherto unreported, mainly because most common methods available for the determination of chlorophyll in the leaf were made in inorganic solvents (Arnon 1949; Winterman and De Mots 1965). Since the anatomical structure of the leaf near the abaxial and adaxial is quite different it is logical to assume that the chlorophyll content on the two sides might be different in most of the dicotyledonous leaves. Because of lack of suitable method for *in vivo* estimation, quantitative information on this aspect is not available. In this communication the recent technique of photoacoustic spectroscopy has been applied for the relative amount of chlorophyll a in the two sides of the leaf.

2. Materials and methods

Photoacoustic effect, though discovered in 1881 by Bell, has recently been used to study the optical properties of solids (Rosencwaig 1975). It has also been used to study the biological samples (Rosencwaig and Pines 1977). In photoacoustic spectroscopy chopped or modulated monochromatic light is allowed to fall on a sample surrounded by non-absorbing inert gas in an enclosed cell due to which periodic light absorption occurs. These excited molecules decay non-radiatively producing periodic heat flow to the surface of the sample. Heat transfer from the sample surface to the surrounding gas generates pressure fluctuations which are detected by sensitive microphones. The acoustic signal is then normalised against the signal of carbon black to compensate for the variation of power output of the light source. Plotting the normalised photoacoustic (PA) signal as a function of wavelength produces a PA spectrum which exhibits

the characteristic absorption peaks and non-radiative decay processes of the sample. Since the strength of the PA signal is closely related to the amount of light absorbed and since only absorbed light can produce an acoustic signal this method has been used to study chlorophyll content under *in vivo* condition on the two sides of the plant leaf. PA spectra were recorded (using Prinston Applied Research Model 6001 instrument), corrected for blank absorption and normalized using carbon black as reference.

Leaves of plants fully grown under natural (*ca* 12 hr) photoperiod (temperature about 35°C day and 22°C night) were thoroughly washed with distilled water and dried under pressed condition for more than 24 hr. The cut sample of the leaf (6 × 4 mm), was scanned in the range of 500 to 700 nm. To reduce noise, the spectra were noted after two scans.

3. Results and discussion

Pennisetum typhoides, a C_4 monocot plant, NADP-ME type was used to study variation of chlorophyll content from leaves in different positions. PA spectra for I, II and III leaf under dry condition are given in figure 1. It is seen that the chlorophyll a content increases monotonically from I to III leaf. This phenomenon has also been observed on various other plants (Sestak 1977). This indicates that PA spectroscopy gives results

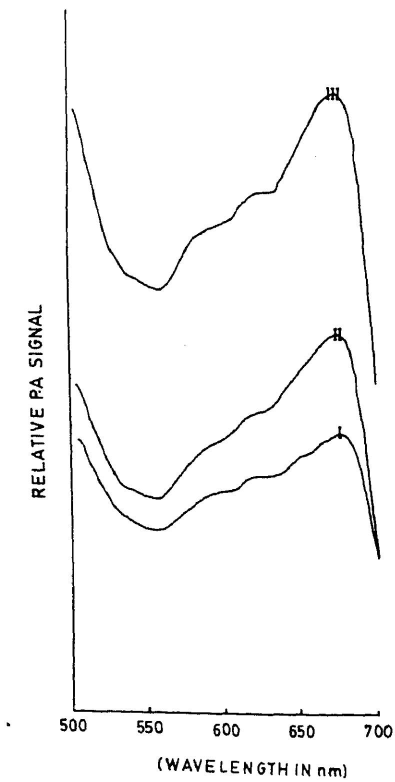

Figure 1. PA spectra of chlorophyll a on adaxial side of I, II and III leaf (from the top) of *Pennisetum typhoides*.

similar to those obtained using other conventional methods. However, PA spectra recorded for two different sides of these leaves gave the abaxial to adaxial ratio of chlorophyll a (R) as 1·13, 1·26, 1·05 and 1·05 respectively for I, II, III and matured leaf. These results suggest that chlorophyll a distribution is not symmetrical during the early development of the leaf and probably the cell distribution is isobilateral only when the leaves are matured.

The PA spectra for the different parts of same leaf, base, middle and tip are given in figure 2(a) for *Amaranthus hypochondriacus*, a C_4 plant. These spectra show that the amount of chlorophyll a increases from the tip to the bottom of the leaf and agrees with the results obtained using Arnon's method in our laboratory. Figure 2(b) gives the PA spectra using the same leaf from midrib to margin at base. These spectra also show that chlorophyll a content decreases from mid to margin. Similar results were obtained when Arnon's method was followed. It is interesting to note that the R value does not change monotonically as one goes from tip to base or midrib to margin.

Results of the two experiments discussed above show that the R value varies from species to species as well in different parts of the same leaf. In order to get some insight

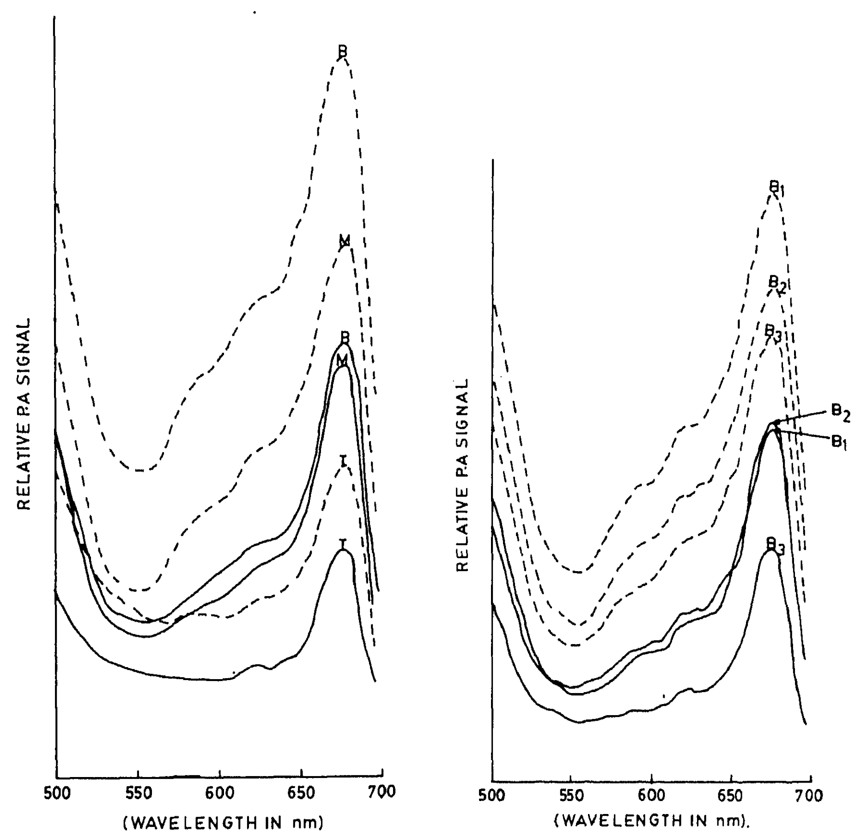

Figure 2. PA spectra of chlorophyll a of *Amaranthus hypochondriacus* from **a**. Tip to base in two sides of the leaf. **b**. Midrib to margin at the base of the leaf. (-----) abaxial side. (————) adaxial side. (T = tip; M = middle; b = base; B_1 = midrib; B_2 = middle of midrib and margin; B_3 = margin).

Table 1. Abaxial to adaxial ratio of chlorophyll a (R) for the leaves of various plants (Fully mature leaves were scanned and R value calculated)

Plant	R value	Plant	R value
Monocots		Dicots	
C_4 *plants:*		C_4 *plants:*	
NADP-ME type		NADP-ME Type	
Panicum antidotale, Retz.	1	*Alternanthra pungens*, HBK.	1·66
Pennisetum purpureum, Rich.	1	*Cleome gynandra* DC.	1·26
Pennisetum typhoides, Stampf and Hubb.	1·05	*Euphorbia hirta*, L.	0·87
Sorghum vulgare, Moench.	1·03	*Gomphrena decumbens*, Jacq.	0·83
Zea mays, L.	0·92	NAD-ME Type	
NAD-ME type		*Amaranthus edulis*, L.	1·14
Panicum coloratum L.	0·65	*Amaranthus hypochondriacus*, L.	1·24
PEP-CK type		C_3 *plants:*	
Chloris gayana	1·02	*Arachis hypogaea*, L.	0·49
Panicum maximum, Jacq.	1	*Cassia siamea*, Lam.	0·81
C_3 *plants:*		*Cleome viscosa*, L.	1·53
Avena sativa, L.	0·9	*Corchorus olitorius*, L.	0·91
Hordeum vulgare, L.	0·9	*Achanthospermum hispidum* DC	1·12
Oryza sativa, L.	1·1	*Tridax procumbens*, L.	1·38
Triticum aestivum, L.	1·02	*Achyranthes aspera*, L.	1·18
		Dolichos lablab, L.	1·21
		Waltheria indica, L.	0·85

into this problem we have scanned both monocots and dicots of C_3 and C_4 plants. In C_4 category we have studied plants which fall under NAD-ME, NADP-ME and PEP-CK type and the results are summarised in table 1. The R value for monocots studied here is fairly constant and near unity (table 1). Thus for the plants having R value different from unity the chlorophyll a distribution on the two sides of the leaf is asymmetrical.

The R value is > 1 for *Alternanthera pungens* and *Cleome gynandra* but < 1 for *Euphorbia hirta* and *Gomphrena decumbens*. Same trend was observed for C_3 dicots studied. The orientation of leaves with respect to ground and sun position was also studied and it was found that there was no correlation with the R value.

These studies suggest that the R value is not correlated with any of the taxonomic classifications of plants and depends presumably on the anatomical structure of the leaf. The present studies indicate that abaxial to adaxial ratio of chlorophyll a (R) has certain significance in photosynthetic process and requires an indepth study to understand the asymmetry of distribution of chlorophyll a in the leaf.

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Psychoactive plants in need of chemical and pharmacological study

RICHARD EVANS SCHULTES
Botanical Museum of Harvard University, Cambridge, Massachussetts, USA

Abstract. Reports on the inebriating properties of plants—some employed in magico-religious ceremonies of primitive societies in various parts of the world—continue to appear. Their sources are many: reports of travellers, anthropological writings, historical documents, herbarium specimens and others. The diversity and wide occurrence of the reports have tended to keep them from the scrutiny of investigators who might have studied the plants for the ascertainment of their active principles.

In an effort to focus attention on some of these presumably psychoactive plants, the following notes are offered. Only those species which seem most urgently in need of attention are listed. There are others which appear to be promising albeit not of such immediate interest because of the vagueness of the ethnobotanical reports of their use or because of extreme difficulty in procuring sufficient supplies of the plant for phytochemical study. Even though the number of species listed below is limited, it is obvious how much remains to be done in the interdisciplinary study of biodynamic plants.

A very recent survey of natural hallucinogens has pointed out that more than 200 species of higher plants comprise the study, that they are widely distributed in the plant kingdom (146 genera in more than 50 families) and that the active principles are known for only about 45 species (Schültes and Farnsworth 1980) *Bot. Mus. Leafl., Harvard Univ.* **28** (186–190). This survey attributes the lack of chemical knowledge of these plants to two causes: (i) the lack of good animal models which the chemist can utilize in monitoring his isolation work; and (ii) the paucity of field work of scientific trustworthiness in fast disappearing aboriginal societies. The survey ends with the statement that the "... Plant kingdom remains a fertile and almost virgin territory for those interested in the discovery of new psychoactive drugs, not to mention other types of biologically active compounds waiting in silent hiding."

The extreme paucity of phytochemical studies on these plants of very significant use in primitive societies emphasizes one of the most important results from ethnobotanical investigations: the ability to orient chemical analyses along lines of biodynamically useful species.

Keywords. Psychoactive plants; Dictyonemataceae; Lycoperdaceae; Gramineae; Cyperaceae; Araceae; Amaryllidaceae; Moraceae; Zingiberaceae; Orchidaceae; Fumariaceae; Leguminosae; Rutaceae; Malpighiaceae; Coriariaceae; Sapindaceae; Malvaceae; Cactaceae; Ericaceae; Desfontainiaceae; Labiatae; Solanaceae; Acanthaceae; Bignoniaceae; Rubiceae; Campanulaceae; Compositae

1. Dictyonemataceae

Dictyonema sp.

The Waorani Indians of Amazonian Ecuador have two hallucinogens: *Banisteriopsis muricata* and a species of the basidiolichen genus *Dictyonema* (Davis and Yost 1983 *Bot. Mus. Leafl., Harvard Univ.* **29** 291–295). The name in Waorani is *nɇnɇndapɇ*, a term that they apply to many fungi.

It has been suggested that this lichen represents an undescribed species.

The *Dictyonema* was once employed in shamanistic ritual, but it apparently has dropped out of use. It was taken "when bad shaman ate it to send a curse to cause other Waorani to die." It was prepared as an infusion with certain species of bryophytes called

kigiwai. The effects of the drug were headache and confusion; it is said also to cause sterility and may be given to children to induce barrenness.

Nothing is known of the chemical constituents of *Dictyonema*, and the authors cited above state that "it may be difficult to gather adequate supplies for analysis; so rare is this species in Waorani land that one of us (JY) heard references to it for over seven years before encountering it in the forest."

2. Lycoperdaceae

Lycoperdon marginatum *Vittadini, Monogr. Lycoperd.* (1843) plate 1, figure 11.
Lycoperdon mixtecorum *Heim* in *Comptes Rend.* **254** (1962) 789; in *Rev. Mycol.* **31** (1966) 156.
Tarahumare medicine men in northern Mexico take puffballs, which they call *kalamoto*, to make it possible to approach people without being seen and in order to cause sickness (Bye: in Díaz: 1976, *Cuad. Cient. Cemef.* (*Mexico*), no. 4 49–72). The Mixtecs of Oaxaca in southern Mexico use two species of *Lycoperdon*—*L. marginatum* and *L. mixtecorum*—to induce a condition of half-sleep, during which they experience auditory hallucinations (Ravicz 1960, 1961, *An. Inst. Nac. Antrop. Hist.* **13** 73–92; Heim: *Nouvelles Investigations sur les Champignons Hallucinogènes*, Edit. Mus. Nat. Hist. Nat. Paris, 1967 195–199).

No psychoactive principles are known from the genus *Lycoperdon*.

3. Gramineae

Cymbopogon densiflorus *Stapf* in Prain, *Fl. Trop. Afr.* **9** (1918) 289.
The flower, smoked either alone or with tobacco by witch doctors in Tanganyika, "causes dreams" which are believed to foretell the future. Native medicine men in Tanganyika are said to employ this grass as an intoxicant (Herbarium collection: *Newbould et Hartley 4319*).

Active principles are as yet unknown from the genus *Cymbopogon*.

4. Cyperaceae

Scirpus sp.
In northern Mexico, the Tarahumare fear *bakana*, a species of *Scirpus*. It is believed to cure insanity, and the whole plant is thought to protect the mentally ill. These Indians hold that the intoxication induced by this sedge permits them to travel over great distances to speak with the ancestors. Brilliantly coloured visual hallucinations are induced (Bye 1975 in Diaz: *Cuad. Cient. Cemef.* **4** 61–62).

Harmala alkaloids have been reported from *Scirpus* and the related genus *Cyperus* (Raffauf: *A Handbook of Alkaloids and Alkaloid-bearing Plants*, Wiley-Interscience, New York (1970)); Willaman and Schubert: *Alkaloid-bearing Plants and their Contained Alkaloids*, 1961, U.S. Govt. Printing Office, Washington, D.C. 80; 1970 Willaman and Li: *Lloydia* 33, no. 3A.

5. Araceae

Acorus Calamus *Linnaeus*, Sp. Pl. (1753) 324.
The Cree Indians of northwestern Canada are believed to chew the root of this plant for its hallucinogenic effects. It can induce hallucinogenic conditions in excessive doses.

While it has been suggested that α-asarone and β-asarone, which have structural resemblances with mescaline, may be responsible for the psychoactivity, there is as yet apparently no evidence that asarone can experimentally be associated with hallucinogenic activity (1967, Hoffer and Osmund: *The Hallucinogens*, Academic Press, New York, 55–56).

6. Amaryllidaceae

Pancratium trianthum *Herbert* in Ann. Nat. Hist., ser. 1, 4 (1840) 28.
This perennial plant, known to the Bushmen of Dobe, Botswana, as *kwashi* is believed to be psychoactive (1970, Schultes: *Bull. Narcotics* **22,** pt. 1 25–53). These tribesmen cut the bulb and rub it over incisions or scarifications on the head to induce visual hallucinations.

Some of the 15 species of *Pancratium* possess toxic principles, chiefly alkaloids. Cardiac poisons are known from the genus, but no truly hallucinogenic constituents have been isolated from *P. trianthum* (Schultes et Farnsworth: 1980 *Bot. Mus. Leafl.*, Harvard Univ. 28 175).

7. Moraceae

Helicostylis tomentosa (*P.* et *E.*) Rusby in *Mem. Torr. Bot. Club* **6** (1896) 120.
Helicostylis pedunculata *Benoist* in *Bull. Mus. Hist. Nat. Paris* **25** (1919) 298.
Takini is a Karib Indian name for several species of *Helicostylis* in the Guianas. It is reported that the Indians and bush Negroes of Surinam employ the reddish sap or latex of the bark, which is mildly toxic, as an hallucinogen in ceremonial witchcraft. The identity of the source tree has not yet been definitively settled, but it is presumed that these two species of *Helicostylis* may be utilized (Stahel: *Del Nuttige Planten van Suriname.* Bull. no. 59, Dept. Landbouwproef-station, Suriname, 1944; Ostendorf: *Nuttige Planten en Sierplanten in Suriname.* Bull. no. 79, Landbouwproefstation Suriname, 1962; Kloos: *The Maroni River Caribs of Surinam.* Van Gorcum and Comp., n.v., Assen, Holland, 1971; Lindeman and Mennega: *Bomenboek voor Suriname*, Dienst's Lands Bosbeheer Suriname, Paramaibo, Surinam, 1963). The source tree has also been variously attributed to the genera *Piratinera* or *Brosimum* of the Moraceae (1969, Hegnauer: *Chemotaxonomie der Pflanzen*, Birkhäuser Verlag, Basle, **5** 109) or even to the euphorbiaceous *Pausandra* (de Goeje: *Philosophy, Initiation and Myths of the Indians of Guiana and Adjacent Countries*, Internationales Archiv für Ethnographie, Leiden, 1943).

Extracts of the bark of both of these species of *Helicostylis* have elicited central nervous system depressant effects in animals similar to those expected from *Cannabis*, but the actual active chemical constituents remain to be characterized (Schultes and Farnsworth: loc. cit., 183–184).

Maquira sclerophylla (*Ducke*) *C. C. Berg* in 1969, *Acta Bot. Neerl.* **18** 463.
An hallucinogenic snuff is said formerly to have been prepared from the fruits of the gigantic forest tree *Maquira sclerophylla* (figure 1). It was employed ritualistically in dances in the Pariana region of the Brazilian Amazon, where the snuff was known as *rapé dos indios* (Schultes: 1963 *Harvard Rev.* **1**, no. 4 26; 1963, *Psych. Rev.* **1**, no. 2 158).

This tree of the Amazon forest was known formerly as *Olmedioperebea sclerophylla* Ducke.

No chemical studies of the fruits have apparently been published, nor is there absolute certainty that the snuff was made from this fruit alone or with other plant

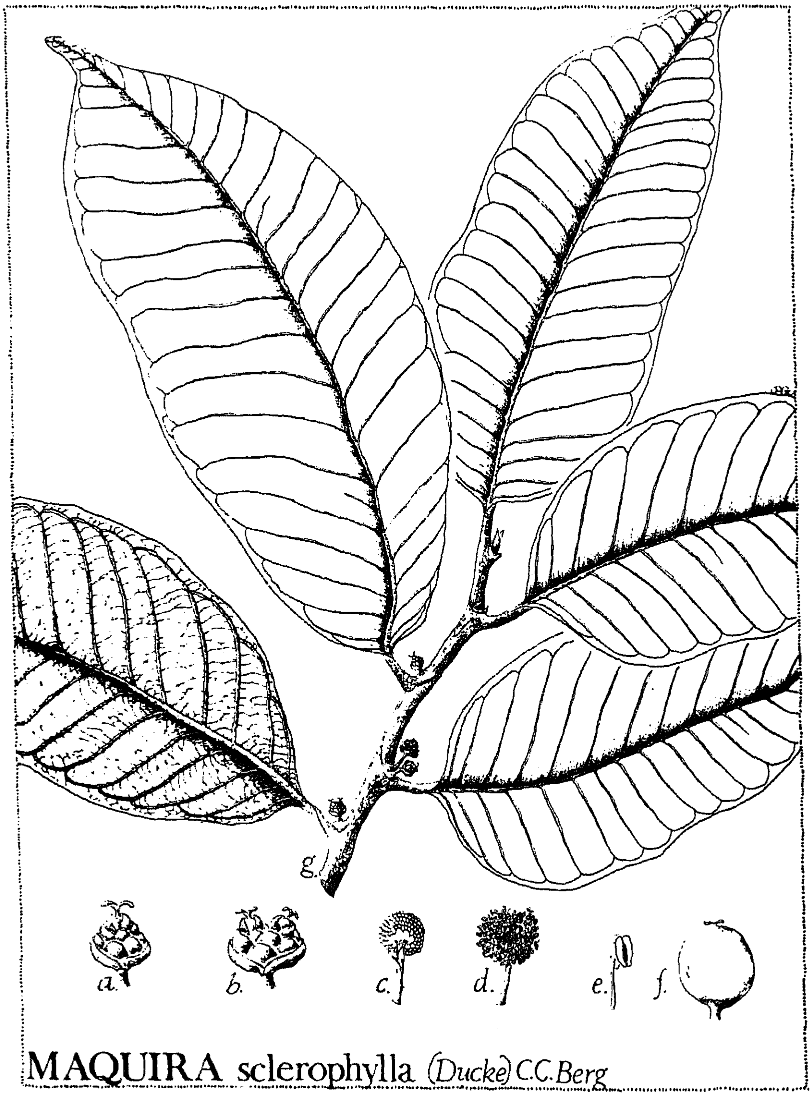

Figure 1.

only the very aged now know how to prepare the drug.

Morus rubra *Linnaeus*, Sp. Pl. (1773) 986.

The Red Mulberry of North America is well-known as the source of a delicious fruit, but it has been reported (J. Hardin and J. Arena: *Human Poisoning from Native Cultivated Plants*, Duke Univ. Press, Durham, N. C. (1969) 70) that the unripe fruits and a milky sap in the leaves and stems can induce hallucinations and may stimulate the nervous system, causing stomach upset.

8. Zingiberaceae

Kaempferia Galanga *Linnaeus*, Sp. Pl. (1753) 3.

There are vague reports of the hallucinogenic use of *maraba* (*Kaempferia Galanga*) in New Guinea (1962, Barrau: *J. Agric. Trop. Bot. Appl.* **9** 245–249).

The rhizome of this plant is rich in essential oils, but psychoactive principles have not yet been reported (Hegnauer: 1963 loc. cit. 2 461).

9. Orchidaceae

Oncidium Cebolleta (*Jacq.*) *Swartz* in *Vet. Akad. Handl. Stockh.* **21** (1800) 240.

This epiphytic orchid is said to be valued by the Tarahumare Indians of Mexico as an hallucinogen, employed when there is no supply available of the *peyote* cactus (*Lophophora Williamsii* (Lem.)) Coult. (Bye: loc. cit.).

An alkaloid of unknown structure has been reported from *Oncidium Cebolleta* (Willaman and Li: loc. cit. 151).

10. Himantandraceae

Homalomena *sp.*

The natives of Papua are said to eat the leaves of a species of *Homalomena*, called *ereriba*, with the leaves and bark of *Galbulimima Belgraveana* (F. Muell.) Sprague to induce a narcosis characterized by a violent and crazed condition followed by deep sleep; they see and dream about men and animals which they are to kill (1958, Barrau: *J. Agric. Trop. Bot. Appl.* **5** 377–378).

No toxic substance is known from *Homalomena*, although 28 alkaloids have been isolated from the bark of *Galbulimima Belgraveana*; there is no indication, however, that these alkaloids are hallucinogenic (Ritchie and Taylor in 1967, Manske and Holmes (eds.): *The Alkaloids*, Academic Press, New York **9** 529–543).

11. Annonaceae

Annona palustris *Linnaeus*, sp. Pl. (Ed. 2, 1762) 757.

It has been reported that the fruit of *Annona palustris* of the West Indies is usually not eaten because it has "strong narcotic properties" (C. Knight: 1829 *The Library of Entertaining Knowledge* Charles Knight, London 385–386).

12. Gomortegaceae

Gomortega Keule (*Mol.*) *I. M. Johnston* in *Contrib. Herb.*, n.s. 3, no. 70 (1924) 92.
Lucuma Keule Molina, Sagg. Chile (1782) 187.

One of the presumably psychoactive plants of South America most imperfectly understood is *Gomortega Keule.*

Because of its unique and limited geographical range as a strict endemic of central Chile, and its phylogenetic position as a monogeneric family—Gomortegaceae—is an interesting order—Ranales—it is urgent that we learn more about the plant. Most important of all: its role as an intoxicant, now probably no longer extant, calls for phytochemical corroboration.

An ethnobotanical study of this plant has recently been published (Plowman: 1971 *Bot. Mus. Leafl.*, Harvard Univ. 22 61–92).

In 1965, Mariani-Ramírez reported that the Mapuche Indians, who call *Gomortega Keule hual-hual* or *keule*, formerly valued this tree as a narcotic (Mariani-Ramírez C.: *Temas de Hipnosis.* Editorial Andres Bello, Santiago, Chile (1965) 357–358). The intoxicating effects may or may not have been hallucinogenic. The fruits, especially in the fresh state, are said to be inebriating.

A search of the literature and several herbaria, however, give little evidence that the plant is in any way intoxicating. Of the several specimens at Kew, for example, one was sent in by *W. W. McKay* for naming in 1911: it bears the following information—"A fine, large, handsome spreading tree with glossy leaves, flowers inconspicuous; fruit edible, yellowish when ripe; native name Queille; grows near Concepcion in a dry situation." A specimen in the Arnold Arboretum (*A. Santa Cruz* s.n.) collected in 1938 states "Frutos comestibles (cocidos) que se venden con alguna frecuencia en los mercados de la ciudad de Concepción. Quele"; and one in the Gray Herbarium, also from Concepción (*J. W. Walker 207*) reports: "Tree *ca.* 25 feet tall. Leaves when crushed aromatic (like turpentine). Mature fruit light yellow. Wood white-reddish, rapidly turning pink when cut. Common."

Ruiz and Pavón, who found this tree in Chile during their botanical expedition to Chile and Peru (1777–1788), wrote the following concerning the plant: Ruiz: 1951 *Relación Histórica* . . . [Ed. J. Jaramillo—Arango] Real Academia de Ciéncias Exactas, Físicas y Naturales de Madrid 1 234–235 "*Gomortega nitida*: v. *keule.* This is the tallest, most leafy and attractive tree that, after the *pino de Chile*, grows in this country. It can be distinguished from afar from the other trees on account of the greenness and beautiful luster of its leaves. Its trunks yield fine woods of a dark red hue with an excellent glossiness after being carefully sanded. Its leaves have an acid-astringent taste. They stick to the teeth when they are chewed because of their high content of resin; when rubbed between the fingers, they give off an odour like that of rosemary and spirits of turpentine, from which we may infer that the plant has efficaceous corroborative and soothing virtues; the leaves burn easily in the fire, even when they are green. The beautiful fruits are the size of small hen's eggs, glossy and yellow and invite one to eat them. But when they are eaten in excess, they bring on headache; the pulp, although not very juicy, is rather sweet and tasty. And the nut or pit is as hard as a rock; the rind is very thick and encloses two or three seeds in as many small compartments. This tree is evergreen and flowers or fruits the year round, and it regularly flowers again when the fruit ripens or near that time. The natives state that there is another species of *keule* in the forests between Arauco and Valdivia; but I have

not seen more than the cases of the fruit which are more pointed at one end and smaller than those of the first species mentioned."

Almost nothing is known about the chemistry of *Gomortega Keule*. In the mesophyll and in the bark there are aromatic oil glands and deposits of calcium oxalate in the form of small needles and prisms (Hegnauer: 1966 loc. cit. 4 210). The leaves have apparently high concentrations of essential oils (Schultes et Hofmann 1980 *The Botany and Chemistry of Hallucinogens*, (ed. 2), 334).

The family belongs to an order—Ranales—rich in essential oils. It appears to be related to Monimiaceae and Lauraceae.

It is interesting to note that Philippi (*Elementos de Botánica para el Uso de los Estudiantes de Medicina i Farmácia en Chile* (1869)) wrote the following, "The *Queule* is placed with the Lauraceae, *Adenostemum nitidum* Pers., a tree growing from Nuble to Queule River, and the leaves and flowers of which look much like those of *Peumo* a species of *Laurus*. Its fruits, yellow drupes four centimetres in length, are good for making jellies. But I have observed that the fruit is trilocular and that the seed has a very large albumen—important characters that do not permit us to leave *Queule* associated with the Lauraceae; probably it should form a special family."

Murillo, in his *Plantes Médicinales du Chili*, A. Roger and F. Chernoriz, Paris (1889), does not mention *Gomortega Keule*.

Tropane alkaloids have been reported from *Gomortega Keule* (Bodendorff and Kummer 1962 *Pharmaz. Zentralhalle Deutschl.* 101 620–622; Plowman: loc. cit.).

13. Fumariaceae

Fumaria media *Loisel* in 1809, Desv., *J. Bot.* 2 357.
This species, native to Mediterranean Europe, is believed in Chile to have sedative and hypnotic properties. In Chile, it is called *hierba de la culebra* ("snake plant") or *hiel de la tierra* ("earth gall") (Mariani R.: loc. cit. 358–359).

The common fumitory, *Fumaria officinalia*, af. Europe, has protopine and seven other alkaloids (Raffauf: loc. cit.). Apparently, analyses of *F. media* have not been published.

14. Leguminosae

Caesalpinia sepiaria *Roxburgh*, Hortic. Beng. (1814) 32.
In China, this plant—known as *yün-shih* (figure 2)—is reported as an hallucinogen. An early Chinese herbal, the Pen-ts'-ao-ching, states that the "flowers could enable one to see spirits and, when taken to excess, cause one to stagger madly." And they may even cause, when ingested over a period, levitation and "communication with the spirits" (Li: 1977 *Bot. Mus. Leafl., Harvard Univ.* **25** 161–181).

An alkaloid has been isolated from *Caesalpinia sepiaria*, but its structure and physiological activity are still unknown (Raffauf: loc. cit.).

Canavalia maritima (*Aubl.*) *Petit-Thouars* in Desvaux, *J. Bot.* **1** (1813) 80.
On the Gulf Coast of Mexico, this plant is reputedly often substituted for *Cannabis sativa* L. While seeds have been found in graves in southern Mexico, there is no report of its use in our cultures. In Peru, remains of this plant have been found in sites dated from 300 to 900 A.D. (Díaz: loc. cit. 181).

Figure 2. Yün-shih or *Ceasalpinia sepiaria* (Chêng-lei-pên-tsao, 1249 ed.) (from Lin-Li 1977).

L-betonicine has been found in *Canavalia maritima*, but this compound is apparently not hallucinogenic (Díaz: loc. cit. 181; Raffauf; loc. cit.)

Cytisus canariensis (Li.) *O. Kuntze*, *Rev. Gen. Pl.* 1 (1891) 177.
Native to the Canary Islands, this shrub has been widely distributed as an ornamental. Its hallucinogenic use by the Yaqui medicine men of northern Mexico has been documented (Fadiman 1965 Econ. Bot. 19 383).

Cytisine, common in this family, occurs in *Cytisus canariensis*, but there is no certainty that this alkaloid is truly hallucinogenic. Further chemical studies are warranted.

Piscidia carthaginensis *Jacquin*, Enum. Pl. Carib. (1762) 27.
This plant is known in Martinique as *bois enivrant* ("intoxicating wood"). The use of the leaves crushed and thrown into water as a fish poison is well recognized. Less well-known, however, are its narcotic properties. The fruit, according to the collector, has a "tonic and narcotic principle" (Herbarium collection: *P. Duss 120b*).

Active principles have not yet been reported from the genus *Piscidia*.

Zornia latifolia *De Candolle*, Prodr. 2 (1825) 317.
The leaves of *Zornia latifolia*, known in Brazil as *maconha brava* ("wild marijuana"), are dried and smoked as an "hallucinogenic substitute for *Cannabis*" (Herbarium collection: *Prance, Rodrigues et al* 8917).

No psychoactive constituent is as yet known from *Zornia*.

Sophora secundiflora (*Ort.*) *Lagasca ex DeCandolle*, Cat. Hortic. Monsp. (1813) 148.
This beautiful shrub (figures 3 and 4) is native to the dry American Southwest and

Figure 3.

northern Mexico. It is known in the US as *mescal bean* or *red bean*; in Mexico as *frijolillo*. Indians of the region formerly ingested the bean in the ceremonial Red Bean Dance as an oracular and divinitory medium and for inducing visions in initiatory rites (LaBarre 1964) *The Peyote Cult*. Shoe String Press, Inc., Hamden, Conn. 126–127). Its use died out in the *US* with the arrival of the much safer narcotic peyote *Lophophora Williamsii* (Lem.) Coult.

Mescal beans contain cytisine, but real hallucinogenic effects are not caused by this alkaloid (Zachowski 1938 *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* **189**:

Figure 4. *Sophora secundiflora.*

327–344). Further chemical studies may indicate the presence of other psychoactive constituents.

There are historical reports that go back to 1539 (Schultes and Hofmann: loc. cit. 157). Archaeological remains of the beans in a context suggesting ritualistic use are dated earlier than 1000 A.D. (Adovasio and Fry: 1976 *Econ. Bot.* **30** 94–96).

15. Rutaceae

Amyris demifera *Linnaeus*, Syst. (Ed. 10, 1759) 1000.
Matthiessen (in Audubon Mag. (1972) 30) suggests the following: "Perhaps the aromatic torchwood [*Amyris elemifera*] is an unrecognized hallucinogen: Russell Niedhauk, an accomplished woodworker, speaks of strange effects from a few minutes of carving."

Sesquiterpenes have been found in the genus *Amyris* (1974, Gibbs: *Chemotaxonomy of Flowering Plants* 3 1671).

16. Malpighiaceae

Banisteriopsis muricata (*Cav.*) *Cuatrecasas* in *Webbia* 13, no. 2 (1958) 490.
Several species of *Banisteriopsis* have long been known as the basis for an hallucinogenic drink of much of tropical South America variously called *caapi*, *ayahuasca*, *yajé*, *natema* or pindé. It is prepared usually from the bark of *B. Caapi* (Spr. ex Griseb.)

Morton or *B. inebrians* Morton, both of which contain β-carboline alkaloids.

Banisteriopsis muricata is the species employed by the Waorani Indians of Amazonian Ecuador (Davis and Yost 1983: loc. cit. **29** 291–295). This forest liana and the drink prepared from it are known to these Indians as *mii*. It is taken by shamans to call upon the *wenae* (malevolent spirits) to wreak evil on an enemy. The taking of *mii* is considered to be an aggressive act; "it may be taken to cure illness but only if prepared by the one who caused the illness (Davis and Yost 1983 loc. cit. **29** 190–191).

This species of *Banisteriopsis* is employed hallucinogenically by other Indians in the Amazon. The Witotos of the Río Ampiyacu in Peru refer to it as *sacha ayahuasca* ("wild ayahuasca") and state that, although weaker in its biodynamic effects than *B. Caapi*, it can be used in the same way.

17. Coriariaceae

Coriaria thymifolia *Humboldt et Bonpland ex Willdenow*, Sp. Pl. 4, pt. 2 (1806) 819. This high Andean shrub, known in Colombia and Ecuador as *shanshi* (figure 5), has

Figure 5. *Coriaria thymifolia*

long been feared as a virulent poison for browsing animals. The fruits are said to be ingested by Indians in parts of Ecuador to induce an intoxication characterized by sensations of flight (Naranjo: 1969 *Terapia* **24** 5–63).

It has been suggested that the hallucinogenic effects may be due to a glycoside of unknown structure (Naranjo and Naranjo: 1964 *Arch. Criminol. Neuro-Psiquiatr.* Discipl. Conexas **14** 4), but much more chemical study is needed before the psychoactivity of this species is understood.

18. Sapindaceae

Ungnadia speciosa *Endlicher, Atakt. Bot.* (1833) t. 36.
The seeds of the *Mexican buckeye*, considered toxic, may once have been employed as an hallucinogen. They have been found in several archaeological sites in northern Mexico and Texas dating from 7500 B.C. to about 1000 A.D. These caches have been associated with remains of the *mescal bean* (*Sophora secundiflora* (Ort.) Lag.) and *peyote* (*Lophophora Williamsii* (Lem.) Coult.), both hallucinogens, and the seeds of the *Ungnadia* would appear to have been used in a religious context (Adovasio and Fry: loc. cit.).

The seeds of the *Mexican buckeye* contain cyanogenic constituents (Hegnauer: 1973 loc. cit. **6** 279).

19. Malvaceae

Sida acuta *Burman fil.*, Fl. Ind. (1768) 147.
Sida rhombifolia *Linnaeus*, Sp. Pl. (1753) 684.
Mexican natives attribute psychotropic properties to these plants (Díaz: loc. cit. 183–186; in Lozoya: (Ed.) 1976 *Estado Actual del Conocimiento en Plantas Medicinales Mexicanas*, Instituto Mexicano para el Estudio de las Plantas Medicinales, Mexico 10 109–130).

Phytochemical study of this genus is merely in a preliminary stage.

20. Cactaceae

Pachycereus pecten-aboriginum (*Engelm.*) *Britton et Rose* in *Contrib. U.S. Nat. Herb.* 12 (1909) 422.
The Tarahumare Indians of Mexico are said to use this large columnar cactus (figure 6) as a narcotic which they call cawe. What its exact effects are remains doubtful (Pennington: 1963 *The Tarahumar of Mexico—their Environment and Material Culture*, University of Utah, Salt Lake City 166, 167; Bye: loc. cit. 59–60).

This plant contains the alkaloid carnegine or deshydroxypellotine (Agurell: 1969, *Lloydia* **32** 206–210; Bruhn and Lindgren: 1976 *Lloydia* **39** 175–177; Späth: 1929 *Ber. Dtsch. Chem. Gesel.* **62** 1021–1024).

21. Ericaceae

Pernettya furiens (*Hook. ex DC.*) *Klotsch* in *Linnaea* **24** (1851) 83.
Known in Chile as *huedhued*, *tembladerilla* and *hierba loca*, this ericad is said to cause a

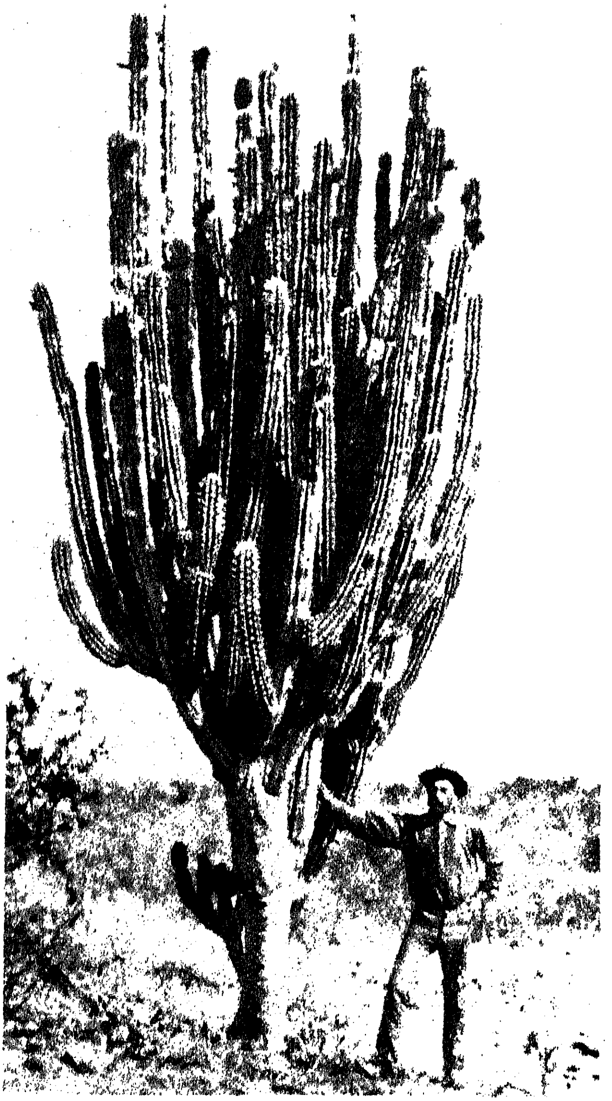

Figure 6. *Pachycereus pecten-aboriginum.* (from Contrib. U.S. Natl. Herb. 1899)

state of mental confusion and delirium similar to that caused by *Datura.*

It appears that chemical studies on *Pernettya furiens* have not been carried out, but the genus is known to be rich in glycosides (Schultes et Hofmann: loc. cit. 356).

Pernettya parvifolia *Bentham,* Pl. Hartw. (1846) 219.
In Ecuador, *Pernettya parvifolia* is called *taglli.* Its fruits, when ingested, induce hallucinations and other psychic and motor alterations (Naranjo: loc. cit. 58–59).

22. Desfontainiaceae

Desfontainia spinosa *Ruiz et Pavón*, Fl. Peru 2 (1799) 47, t. 186.
Known in Chile as *taique*, in Colombia as *borrachero* or *borrachero de páramo*, this high Andean shrub (figure 7) has been reported as an hallucinogen in use by Indians of Chile and of Colombia (Schultes: 1977, *Bot. Mus. Leafl., Harvard Univ.* **25** 99–104).

Although the family Desfontainiaceae seems to be closely related to the alkaloid-rich Loganiaceae, nothing is as yet known of the chemical constitution of *Desfontainia spinosa*.

Figure 7. *Desfontainia spinosa*

Coleus Blumei *Bentham*, Lab. Gen. et Sp. (1832) 56.
Coleus pumilus *Blanco*, Fl. Philip., ed. 1 (1837) 482.
Two species of *Coleus*—*C. Blumei* and *C. pumilus*—are possibly employed in Oaxaca, Mexico, as hallucinogens. Both species are introductions from Asia (Wasson 1962 *Bot. Mus. Leafl., Harvard Univ.* **20** 79).

As yet, no psychoactive constituent has been found in the genus.

Salvia divinorum *Epling et Játiva-M.* in Bot. Mus. Leafl., Harvard Univ. 20 (1962) 75. In the Mazatec country of Oaxaca, Mexico, all natives know and many have a small private plot of *Salvia divinorum* (figure 8) which is vegetatively reproduced. It is apparently a cultigen not known in the wild, an indication perhaps of the great age of its use. The leaves, locally known as *hierba de la Virgin* or *hierba de la Pastora*, are valued

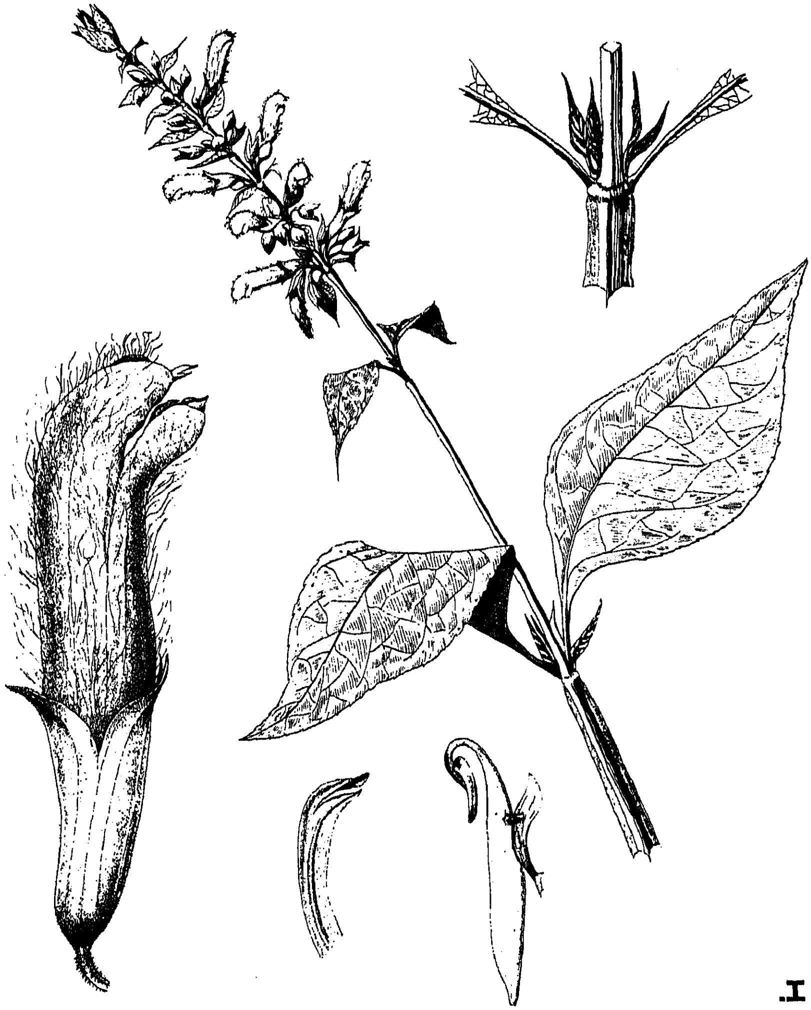

Figure 8. *Salvia divinorum.*

(Wasson: 1962 *Bot. Mus. Leafl., Harvard Univ.* **20** 77–84; Valdes, Diaz and Paul: 1983 *J. Ethnopharm.* 7 287–312).

Scientific investigators have experimentally substantiated the psychoactivity of the leaves, but chemical studies have as yet not disclosed an hallucinogenic principle.

24. Solanaceae

Iochroma fuchsioides (*HBK.*) *Miers* in Hooker, 1848 *Lond. J. Bot.* 7 345.
This beautiful shrub (figure 9) is known in southern Colombia and Ecuador as *arbol de campanilla*, as *borrachera*, *borrachera andake*, *nacedero*, *flor de quinde* ("hummingbird flower"), *panguana* or *guatillo* in Spanish; *totubjansushe* or *tutujansushe* in the Kamsá Indian language. In the Valley of Sibundoy, the bark is made into a tea which, in mild

Figure 9. *Iochroma fuchsioides* in flower and fruit

doses, is purgative, employed especially when there is internal bleeding; in cases of internal injury from severe blows, the root is rasped and eaten raw with salt; the root with other unidentified ingredients may be used as a contraceptive (Bristol: 1965 *Sibundoy Ethnobotany*, Ph.D. thesis, unpubl., Harvard University, Cambridge, Mass).

The plant is valued in preparing a narcotic with hallucinogenic effects. The leaves are simply crushed in water with a handful of freshly rasped bark from the stem; this mixture is then gently heated. The hallucinogenic dose is from one to three cupfuls of the strong decoction over a 3 hr period (Schultes: 1970 *Bull. Morris Arb.* **21** 14; 1977 *J. Psyched. Drugs* **9** 45–49).

The hallucinogenic constituent may be alkaloidal. Raffauf has had positive tests for alkaloids, but merely in preliminary field tests (Bristol: loc. cit.; Schultes: loc. cit.).

The following chemical examination of *Iochroma fuchsioides* is apparently all that is known of the genus on a renin-inhibitor.

"Extracts of *Iochroma fuchsioides* were found to inhibit the enzymatic activity of renin in a fluorometric assay system utilizing a synthetic octapeptide substrate. Subsequent testing in other renin assay systems using the natural protein substrates confirmed this inhibitory activity, although the I values were much less in the plasma systems with natural substrate. The crude aqueous extract gave an I of 5.6 g/ml in the fluorometric assay system. *In vivo* activity was also demonstrated in experiments with rats infused with renin. Intravenous injections of both crude and partially purified preparations lowered the elevated blood pressure of such rats.

"Partial purification of the inhibitor present in aqueous extracts of the plant was accomplished by ammonium sulphate precipitation followed by molecular sieve chromatography on Sephadex G-50. Two active fractions were obtained. The larger molecular weight fraction was not retarded on the Sephadex G-50 column, and assayed only about 40% as active as the crude extract. The smaller molecular weight fraction, which was eluted after about two column volumes, was found to be eight times as active as the starting crude extract."

"The active component in the smaller molecular weight fraction appears to be a heat stable glycopeptide in the 4,000–6,000 molecular weight range, and is anionic at neutral pH. Thus far, attempts at further purification have not been successful." (Miller, Svoboda and Tafur: pers. comm.).

25. Acanthaceae

Justicia spp.

It has been reported that the leaves of several species of *Justicia* are the source of an hallucinogenic snuff amongst the Waika Indians of the northern Brazilian Amazon and adjacent parts of the upper Orinoco basin of Venezuela (Chagnon *et al.*: 1971 *Curr. Anthrop.* **12** 72; Carias-Brewer and Steyermark: 1976 *Econ. Bot.* **30** 57–66). *Justicia pectoralis* (figure 10) Jacq. var. *stenophylla* Leonard is very frequently employed as powdered leaves to mix with the hallucinogenic snuff *epena* or *nyakwana* prepared from red resin-like liquid in the bark of *Virola elongata* (Spr. ex Benth.) Warb. and *V. theiodora* (Spr. ex Benth.) Warb.

Reliable indications of psychoactive principles in *Justicia* are not available, although it has been suspected that tryptamines may be present (Schultes and Holmstedt: 1968, *Rhodora* **70** 113–160).

Figure 10. Waika Indians preparing leaves of *Justicia pectoralis* var *stenophylla* to add to the narcotic snuff nyakwana made basically from the red resin of the bark of *Virola theiodora*

Solanum hypomalacophyllum *Bitter ex Pittier*, Man. Pl. Usual. Venez. (1926) 137. The common name of this plant in Venezuela—*borrachera*—suggests that it is an intoxicant (Herbarium collection: *Gehringer* 2).

It is possible that this species of *Solanum* contains tropane alkaloids so widespread in this family, but an analysis has apparently not been made.

26. Bignoniaceae

Tanaecium nocturnum (*Barb.-Rodr.*) *Bureau et K. Schumann* in Martius, Fl. Bras. 8, pt. 2 (1896) 186.

The Paumarí Indians of the Rio Purus in the Brazilian Amazon prepare a snuff for use by medicine men (figures 11, 12). The green leaves are shredded, wasted, pulverized and sifted through a fine cloth, and the resulting powder is mixed with tobacco snuff

Figure 11. Trunks and leaves of the tropical liana *Tanaecium nocturnum*.

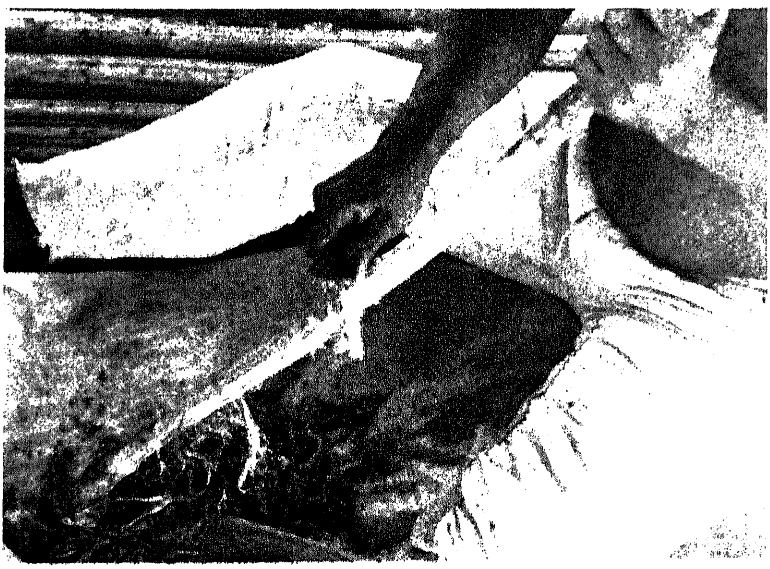

Figure 12. Scraping bark from trunk of *Tanaecium nocturnum* to prepare snuff-paumari Indians, Amazonas, Brazil.

(*Nicotiana Tabacum* L.). This mixture is presumably psychoactive (Prance: 1977, *Econ. Bot.* 31 131–134).

Preliminary chemical examination has indicated a high concentration of hydrogen cyanide in the fresh leaves (Schultes and Hofmann: loc. cit., 360–362).

27. Rubiaceae

Pagamea macrophylla *Spruce ex Bentham* in *J. Linn. Soc.* **1** (1857) 110.
In the Colombian Vaupés, the leaves of this shrub (figure 13) are dried and powdered to

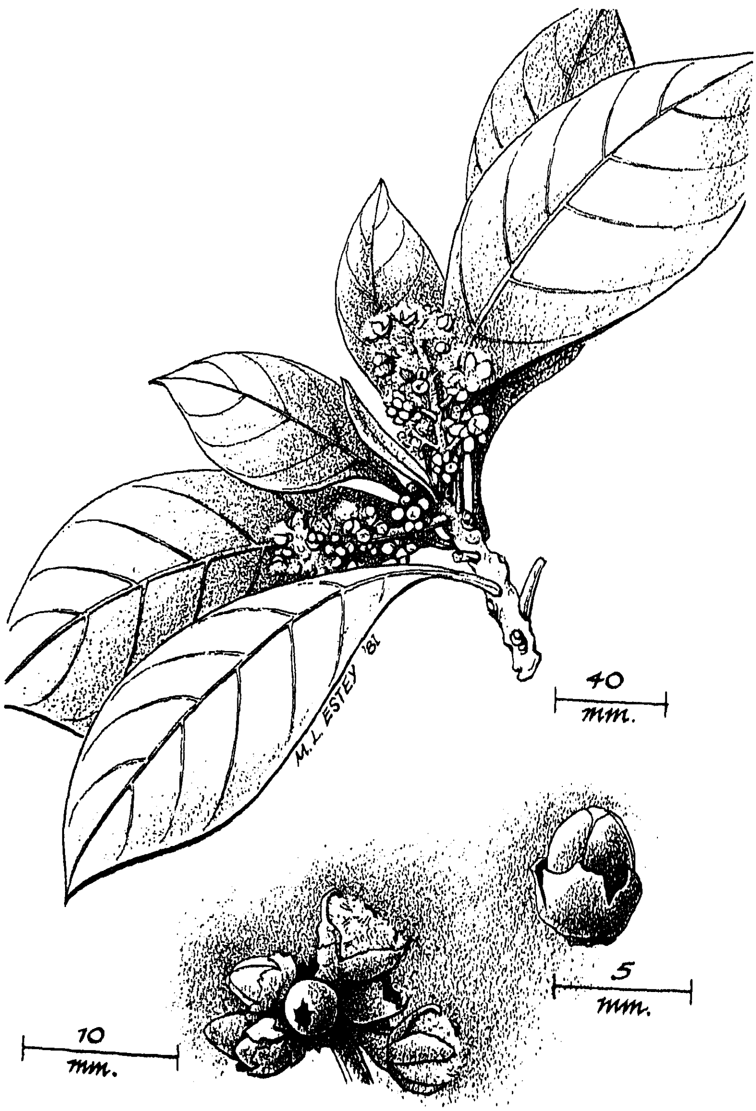

Figure 13. *Pagamea macrophylla* Spr. ex Bth.

prepare a snuff used by Barasana medicine men during curing or divinatory seances (Schultes: 1980 *Bot. Mus. Leafl., Harvard Univ.* **28** 271–275).

No chemical studies have been carried out on the genus *Pagamea.*

28. Campanulaceae

Lobelia Tupa *Linnaeus* Sp. Pl., Ed. 2 (1963) 1318.
This six-foot *Lobelia*, (figure 14) a beautifully majestic plant of the Andes of southern Peru and northern Chile, is widely recognized in Peru as toxic. It is called *tupa* or *tabaco del diablo* ("devil's tobacco") (Ibáñez, J 1955 *Fanerogamia.* Editorial Universitaria. Mariani R.: loc. cit., 363–364).

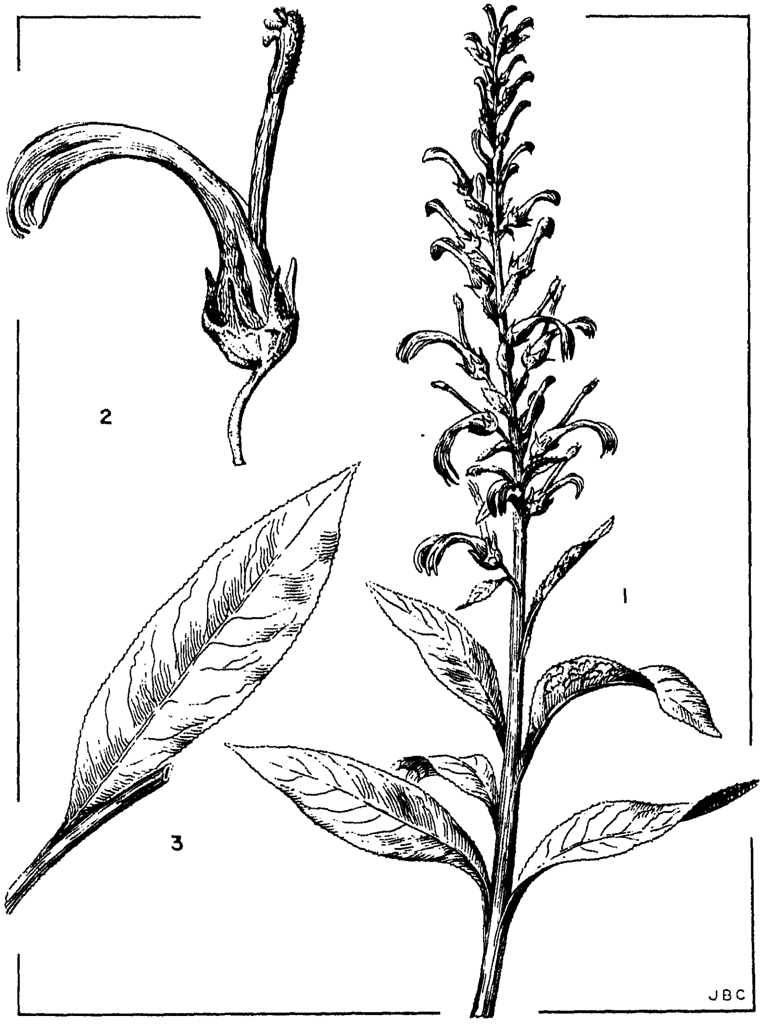

Figure 14. *Lobelia Tupa* L.

Much more remains to be known concerning the ethnobotany of *Lobelia Tupa.* Is it smoked, as the name *tabaco del diablo* might suggest? Does it have a medicinal use? If it is not smoked, how is it used, or is it merely avoided as a toxic species?

Tupa is rich in lobeline and lobeline derivatives which could be responsible for toxicity; but, if the plant is employed narcotically, what is the psychoactive principle (Hegnauer: 1966 loc. cit. **4** 409, 411)? The known constituents could not be responsible for hallucinogenic properties

29. Compositae

Eupatorium solidaginifolium *A. Gray*, Pl. Wright. 1 (1852) 87.
This plant is said to be smoked by the Papago Indian shamans of Arizona in curing seances. The native name of the species is *pihol* (Herbarium collection: *Lipp sin. num.*).

Several alkaloids have been isolated from species of *Eupatorium*, but they do not appear to be psychoactive (Raffauf; loc. cit.).

Calea Zacatechichi *Schlechtendal* in *Linnean* **9** (1834) 589.
This common Mexican shrub is the source amongst the Chontal Indians of a psychoactive preparation. An infusion of the leaves, called *thle-pela-kano* ("leaf of god") is taken to classify the senses and as an aid in divination (MacDougall 1968 *Gard. J.* **18** 105). Whether or not *Calea Zacatechichi* (figure 15) represents a true hallucinogen is open to question. Chemical studies have revealed a large number of polyacetylenes, sesquiterpene lactones, chromenes, triterpenes and flavenoids, but none of these would have hallucinogenic effects (Bohlmann and Zdero: 1977, *Phytochem.* **16** 1065–1068). Preliminary investigation has indicated the presence of a possible new alkaloid (Holmstedt: pers. comm.).

Helichrysum foetidum *Moench*, Method. (1794) 575.
Helichrysum stenopterum *DeCandolle*, Prodr. 6 (1838) 201.
Two species of *Helichrysum* are employed in Zululand by medicine men.

Although coumarins and diterpenes are reported from this genus, psychoactive principles appear to be absent (Hegnauer: 1964 loc. cit., **3** 478 ff.).

Tagetes lucida *Cavanilles*, *Icones* **3** (1795) 33, t. 264.

Known in modern Mexico as *yauhtli* and amongst the Nahuatl as *yahutli*, this marigold, a bushy annual up to 1·5 feet tall was, according to the historian Sahagún, pulverized and the powder was thrown into the faces of captives in the Aztec Empire "to dull their senses" before sacrifice (Díaz in Lozoya: loc. cit. 109–130).

The Huichols call the plant *tumutsali*. They ceremonially smoke a mixture of *Tagetes lucida* and *Nicotiana rustica* L.—a mixture known as *ye-tumutsali*—to induce visions. This smoking often accompanies the taking of peyote (*Lophophora Williamsii* (Lem.) Coult. or *tesguino* (a fermented maize drink) or *cai* (a cactus distillate).

Alkaloids have not been found in *Tagetes lucida*. A number of species have essential oils. Thiophene derivatives and l-inoitol, saponines, tannins, cyanogenic glycosides and coumarines have been reported from the genus (Gibbs: 1974 loc. cit). **2** 1195–1196; Hegnauer: 1964 **3** 497, 526, 528; 1977 Díaz: *Annu. Rev. Pharmacol. Toxicol.* 17 647–675). None of these types of compounds would account for visual hallucinations.

Figure 15. *Calea Zacatechichi* Schlecht.

ichocline reptans (*Wedd.*) *Hieronymus* in Engler, *Bot. Jahrb.* **21** (1896) 369.
the folk medicine of northern Argentina, the woody rhizome of this plant, known as *o*, is smoked and is believed to be an effective cure for stomach ache. Several species called *contra-yerba*, a term indicating a panacea-type medicinal value.

n former times, the root of *coro* was employed "as a narcotic" by the Calchaqui lians in the form of an additive to their alcoholic chicha (fermented beer made with ize); it was considered a strong inebriant. Later references indicate that several tribes Indians of the Chaco smoked the powdered root alone or with tobacco (Zardini: '7 *Bot. Mus. Leafl., Harvard Univ.* **25** 105–107).

Several other species of *Trichocline* are similarly employed and are known in Argentina by the same vernacular name: *T. exscapa* Griseb. and *T. dealbata* Benth. et Hook. fil.

Whether or not the narcotic properties of *coro* are hallucinatory has not been established. Nothing apparently is as yet known of the chemical composition of members of this genus.

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The mitochondrial genome of higher plants

ANDREAS WEIHE and THOMAS BÖRNER

Humboldt-Universität zu Berlin, Sektion Biologie, Bereich Genetik, 1040 Berlin, Invalidenstr. 43, German Democratic Republic

Abstract. The mitochondrial genome of higher plants appears to be the largest known of any life form. Plant mitochondrial (mt) DNA displays substantial intermolecular heterogeneity with respect to size and physical organization. Electron microscopic studies reveal both linear and circular DNA molecules of varying size. Mapping data suggest that a circular master chromosome representing the entire sequence complexity of the genome gives rise to a series of discrete DNA molecules which are generated via recombination processes within certain repeats. Additionally to the large mtDNA, mitochondria of higher plants contain small plasmid-like DNAs behaving, at least in certain cases, like transpositional elements.

Plant mtDNA codes for 18S, 26S and 5S rRNAs and mitochondrial tRNAs as well as for at least 18–20 polypeptides, mainly membrane-bound components of the oxidative phosphorylation system.

Substantial evidence has been accumulated that cytoplasmic male sterility CMS, a maternally inherited trait is controlled by mtDNA. Male fertile and CMS cytoplasms differ in their mtDNA restriction patterns, in the polypeptides synthesized by their mitochondria, and in the appearance and distribution of the plasmid-like small mtDNAs.

Keywords. Plant mitochondrial DNA; mitochondrial genes; plasmid-like mtDNA; cytoplasmic male sterility.

1. Introduction

With the realization that cytoplasmic genes play an important role in plant development, productivity and susceptibility to disease, investigation of the mitochondrial (abbreviated as MT) genome of higher plants has attracted increasing attention. In recent years, analyses on the molecular level of the plant mitochondrial genetic system have revealed a number of interesting and unique features of this genome. In contrast to the animal MT genome, which is strikingly uniform in structure and size (Brown *et al* 1979; Attardi 1981), MT DNA in higher plants displays substantial variability and appears to be the largest known of any life form. Studies to determine the size and physical organization of MT DNA, to identify the number and function of the polypeptides synthesized by the mitochondrion, to map the mitochondrial genome and to elucidate the coordination of nuclear and MT gene expression are now underway in many laboratories. As MT DNA seems to control maternally inherited traits like CMS and susceptibility to certain diseases, progress in this field will be of great importance for plant breeding and production, which holds true also for plasmid-like DNAS discovered in plant mitochondria: they probably could be used as vectors and thus open perspectives for genetic engineering in higher plants.

2. Size and conformation of MT DNA

MT DNA from higher plants contributing from less than 1 % of total DNA in most plant cells up to more than 15 % in muskmelon hypocotyls (Leaver and Gray 1982; Ward *et al*

1981) exhibits a strikingly uniform buoyant density of 1·705–1·707 g/cm³ (Wells and Ingle 1970; Kolodner and Tewari 1972; Vedel and Quetier 1974), from which a G-C content of *ca* 47 % can be concluded. No considerable intramolecular heterogeneity of the G-C content has been detected (Vedel and Quetier 1974). This uniformity in base composition, however, is not reflected in a corresponding physical uniformity. Analyses of plant mitochondria have yielded both circular and linear forms of MT DNA in varying proportion and length. The sizes of mitochondrial genomes range from 40 to 1600 Mdal (see table 1). Even within a single family of plants, the cucurbits, 7–8 fold size

Table 1. Molecular weights of plant mitochondrial DNA.

Plant species	Molecular weight ($\times 10^6$) derived from: contour length measurement[a]	renaturation kinetics	restriction patterns	References
Zea mays	45[b], 36[c], 55[d]	320	388, > 390	Levings *et al* 1979; Ward *et al* 1981; Stern and Lonsdale 1982
Triticum aestivum *Secale cereale* *Hordeum vulgare* *Avena sativa*			140–230, > 150	Quetier and Vedel 1977; Vedel *et al* 1981
Pisum sativum	60–70	74[e], 240	231	Kolodner and Tewari 1972; Ward *et al* 1981
Vicia faba		70[e]	> 120	Kolodner and Tewari 1972; Negruk *et al* 1981
Lupinus luteus			> 118	Augustyniak *et al* 1983
Oenothera berteriana	66		120–130	Brennicke 1980
Brassica sp.			110–140, 144	Vedel *et al* 1982; Palmer and Shields 1984
Parthenocissus tricuspidata	60–70		165	Quetier and Vedel 1977, 1980
Beta vulgaris			265	Powling 1982
Spinacia oleracea	70[e]			Kolodner and Tewari 1972
Nicotiana tabacum		40[e]	206–293	Wong and Wildman 1972; Belliard *et al* 1979
Solanum tuberosum	58–60	100	90	Vedel and Quetier 1974; Quetier and Vedel 1977
Coffea sp.			> 360	Berthou *et al* 1983
Cucumis melo		1600	> 477	
Cucumis sativus		1000	> 497	
Cucurbita pepo		560	475	
Citrullus vulgaris		220	231	Ward *et al* 1981
Lactuca sativa		70[e]		Kolodner and Tewari 1972

[a] Only predominant size classes are enlisted, [b] N-cytoplasm, [c] S-cytoplasm, [d] T-cytoplasm, [e] apparently underestimated values from the early reports of Kolodner and Tewari (1972) and Wong and Wildman (1972)

diversity has been shown (muskmelon 1600 Mdal, cucumber 1000 Mdal, zuccini squash 560 Mdal, watermelon 220 Mdal) (Ward *et al* 1981). The size heterogeneity of plant MT DNA has been demonstrated both by electron microscopy and electrophoretic studies, and these results have been confirmed by DNA renaturation kinetics and restriction endonuclease analysis. The large MT genomes consist mainly of unique sequences (Kolodner and Tewari 1972; Ward *et al* 1981). Evidence for the presence of reiterated sequences representing 5–10% of the MT genome was found in the MT DNA of the cucurbit family (Ward *et al* 1981).

At present, there is no satisfying answer to the question, why plant MT genomes are so large and heterogenous. It has been suggested that plant MT DNA might code for a few additional polypeptides not synthesized in animal mitochondria (see below), but the additional genes never would account for the large portion of excess DNA in plants. If there would be "selfish" DNA present in plant mitochondria, then one would expect considerably higher amounts of repeated sequences, for which no evidence is available. That the excess DNA might serve as a "filler" with yet unknown function has also been discussed (Ward *et al* 1981). At present, it is suggested that in the MT genome of higher plants amplification and rearrangement of sequences has occurred, which has led to nonhomology of the arising DNA molecules and thus created the large variation in size and sequence complexity.

Electron microscopic studies have revealed both linear and circular DNA molecules. In most cases the majority of the molecules is linear, with varying amounts of open circular and covalently closed circular molecules. Contour length measurements have yielded values ranging from 0·5 to 42 μm (Sparks and Dale 1980; Brennicke 1980; Levings *et al* 1979; Quetier and Vedel 1977; Fontarnau and Hernandez-Yago 1982). Plant species with discrete size classes have been described (Kolodner and Tewari 1972; Levings *et al* 1979; Synenki *et al* 1978; Dale *et al* 1981), but in other cases no striking evidence for discrete size classes could be demonstrated (Sparks and Dale 1980; Fontarnau and Hernandez-Yago 1982). In tobacco and bean it has been demonstrated that at least some of the different size classes are dimers of smaller DNA fragments (Dale 1981), whereas in Oenothera, for example, no repeat of a longer sequence could be detected among five size classes (Brennicke and Blanz 1982). A high proportion of circular MT DNA belonging to several size classes is usually obtained from *in vitro* cultured cells (Brennicke 1980; Dale *et al* 1981). The linear molecules of varying length observed in MT DNA preparations have been assumed to be derived by damage during isolation from circular molecules (Levings *et al* 1979), but there is no direct evidence for this. At present, the possibility cannot be ruled out that higher plant mitochondria contain at least part of their DNA in the form of linear molecules. Additionally, in MT DNA preparations minicircles and/or minilinears have been found (Synenki *et al* 1978; Kemble and Bedbrook 1980; Levings *et al* 1979; Sparks and Dale 1980), which may be related to the plasmid-like small MT DNAs identified in the mitochondria of a number of species, *e.g.*, maize, *Vicia faba*, sugarbeet, sorghum, and Brassica (Pring *et al* 1977; Weissinger *et al* 1982; Negruk *et al* 1982; Powling 1981; Pring *et al* 1982; Palmer *et al* 1983) (see below). At present, there are many open questions concerning the general organization of the plant MT genome. One explanation for the presence of many different DNA molecules in the mitochondria of higher plants could be that parts of the MT genome are excised from a "master" molecule, amplified or modified by other events and (in part) recircularized (Dale 1981).

Intermolecular heterogeneity of MT DNA is reflected by restriction analyses. Thus,

none of the observed circular or linear molecules is large enough to account for the high molecular weights deduced from the restriction fragment patterns. Restriction endonuclease patterns of plant MT DNAs are very complex, displaying a large number of fragments of varying stoichiometries. Depending on the restriction endonuclease(s) used, electrophoretic analysis reveals up to more than 50 fragments in a wide variety of plant species (Quetier and Vedel 1977; Pring *et al* 1979; Spruill *et al* 1980; Quetier and Vedel 1980; Powling 1982; Vedel *et al* 1981; Ward *et al* 1981; Boutry and Briquet 1982). In maize, it has been found that most of the fragments generated by Bam H1 restriction do not share homology with other fragments, and the total length of unique fragments exceeds by far the largest circular molecules observed (Quetier and Vedel 1977; Spruill *et al* 1980). These results suggest that plant MT DNAs consist of a series of discrete sequences, which can be located on several genomic elements of different sizes. Restriction endonuclease patterns are strikingly reproducible for a given plant MT DNA. Thus, no differences have been found for MT DNA derived from different tissues of the same plant (Quetier and Vedel 1980) or from subfractions of the mitochondrial population of a given species (Spruill *et al* 1980). This strengthens the assumption that MT DNA heterogeneity is intra- rather than intermitochondrial. Efforts have been undertaken in several laboratories to construct restriction maps of plant genomes. Maps of parts of the MT genome have been published (Chao *et al* 1983; Stern and Lonsdale 1982; Stern *et al* 1982; Hiesel and Brennicke 1983). The first complete restriction map has been reported by Palmer and Shields (1984) for the mitochondrial genome of *Brassica campestris.* Their mapping data are consistent with the following model of mitochondrial DNA organization: The genome is organized into three distinct circular molecules. One of them, the "master" chromosome, contains 218 kb corresponding to the entire sequence complexity of the genome (which is among the smallest known higher plant MT genomes). There are in addition two smaller circles of 135 kb and 83 kb representing two distinct subsets of the master molecule. It is postulated that these two circles interconvert with the largest molecule *via* recombination within a 2 kb repeat, *i.e.* the genome exists in two alternative forms, as the 218 kb large DNA circle or the two smaller 135 kb and 83 kb circles. The recombination within the repeat is speculated to be a site-specific event mediated by a specialized recombination system.

A similar model of organization has been proposed for the much larger MT genome of maize (Lonsdale *et al* 1983). Based on the partial maps of the MT DNA it is suggested that the genome consists of three circular molecules with sizes of approximately 600, 350, and 250 kb. The two smaller circles are thought to arise by intramolecular illegitimate recombination within a 3 kb repeat.

3. Function of the mitochondrial genome

The first MT genes identified in higher plants were those for rRNAs and tRNAs in wheat (Bonen and Gray 1980). 26 S and 18 S rRNA genes are located far apart on the wheat MT genome, which is in common with the situation in maize (Iams and Sinclair 1982) and in fungi (Borst and Grivell 1978), but in contrast to animals (Attardi 1981). Furthermore, genes for a 5S rRNA were identified (no other mitoribosomes contain a 5S rRNA). It could be demonstrated that there exists a close physical linkage of the 5S rRNA genes with those of 18S, but not 26S rRNA (Bonen and Gray 1980). tRNA genes are broadly

distributed over the whole MT genome, with some clustering in the 18S and 5S rRNA gene region. The structural properties of plant mitochondrial tRNAs have not yet been investigated in detail, with the exception of initiator methionine tRNA of wheat mitochondria (Gray and Spencer 1983). The available data suggest that MT tRNAs are totally different complements of the tRNA pool of the plant cell (Guillemaut and Weil 1975; Leaver and Gray 1982). The wheat MT $tRNA_f^{Met}$ gene has been sequenced (Gray and Spencer 1983). Its 3′ end is separated by one basepair from the 5′ end of the 18S rRNA gene. The CCA terminus of the mature tRNA is not encoded in the DNA sequence. The sequence of the wheat initiator tRNA shows similarities to eubacterial and chloroplast $tRNA_f^{Met}$. For rRNA genes more data are available. Thus, it has been shown that the 18S rRNA gene of wheat and maize mitochondria lacks the "Shine-Dalgarno" sequence located near the 3′ terminus of bacterial 16S rRNA (Gray *et al* 1982; Chao *et al* 1983). But in general, there are great similarities between 18S rRNA of plant mitochondria and *E. coli* 16S rRNA in regard to comparative sequence analysis. In wheat, multiple copies of rRNA genes have been suggested, while in maize and faba beans they seem to be confined to a single fragment in different restriction digests (Leaver and Gray 1982; Levings 1983).

The cytochrome *c* oxidase subunit II gene was the first protein coding gene of the plant MT genome thus far identified and sequenced. Using yeast MT DNA specific for the oxi-1 gene (coding for subunit II of cytochrome *c* oxidase), a single 2·4 kb maize MT DNA fragment could be cloned containing two coding regions separated from each other by an intron of 794 base pairs (Fox and Leaver 1981). When compared with the corresponding yeast and bovine genes, the maize MT gene exhibited 47% and 40% homology, respectively. The intron, however, is lacking in yeast and bovine genes and appears to be not even a feature characteristic for plants, since the cytochrome *c* oxidase subunit II gene in the MT genome of *Oenothera*, unlike the maize gene, is not interrupted by an intron (Hiesel and Brennicke 1983). The gene for subunit II of cytochrome *c* oxidase is present only in one Eco R1 fragment of maize MT DNA (Fox and Leaver 1981), which corresponds to the situation in *Oenothera*: the same gene here hybridizes only to one Eco R1 and one Bam H1 fragment of the restricted closed circular MT DNA. When undigested supercoiled DNA from *Oenothera* was probed, however, more than one molecular species contained homologous sequences. Thus, several copies of this gene seem to be organized within different molecules (Hiesel and Brennicke 1983).

The genetic codes of MT DNAs from various organisms differ from the universal genetic code and even from each other (Wallace 1982); the genetic code of higher plant MT DNA, however, seems to be more closely related to the universal code than that of fungal and animal MT DNA. Information available on codon usage in plant mitochondria is based upon the gene for subunit II of cytochrome *c* oxidase in maize and *Oenothera*. In both species this gene does not contain a TGA codon within the open reading frame as do mammalian mitochondrial genomes, where TGA codes for tryptophan. Therefore, it has been suggested that in plant mitochondria this triplet may function as a stop codon. The CGG codon, which is observed in the maize gene at positions, where tryptophan is encoded in the corresponding yeast and beef genes, in *Oenothera* also may code for tryptophan rather than arginine (Leaver and Gray 1982; Hiesel and Brennicke 1983).

Only little is known about the mRNAs in higher plant mitochondria. Hybridization studies with DNA probes from the exon and intron regions of the maize cytochrome *c* oxidase subunit II gene and fractionated MT RNA have revealed that the gene is

transcribed as a higher molecular weight precursor molecule, which is spliced into a major stable RNA of about 2·6 kb, the presumable mature mRNA (Fox and Leaver 1981). There is no evidence for polyadenylated RNA in plant mitochondria (Leaver and Gray 1982).

The mitochondrial polypeptides synthesized by higher plants have been analyzed after protein synthesis by isolated mitochondria. Analyses of the radioactive labelled products of the MT translation by SDS-PAGE and subsequent autoradiography have shown that in a variety of plants, *e.g.* maize, rape and faba beans, a similar set of polypeptides is synthesized by the mitochondria (Leaver and Forde 1980; Forde *et al* 1979; Vedel *et al* 1982; Boutry and Briquet 1982). Major polypeptides (18–20) in the range of 8 to 58 kdal and a number of minor components can be resolved. All of them, except the two higher molecular weight polypeptides, appear to be membrane-bound. Three of these polypeptides have tentatively been identified as subunits I and II of cytochrome *c* oxidase and ATPase proteolipid (subunit 9). The soluble polypeptide of *ca* 44 kdal appears equivalent to the Var 1 ribosomal protein described in yeast and *Neurospora* mitochondria (Leaver and Gray 1982). The other soluble protein synthesized by plant mitochondria has been suggested to be a hexamer of the subunit 9 of ATPase. More recently, also the subunit of MT F_1-ATPase was demonstrated to be synthesized in isolated mitochondria of *Vicia faba* (Boutry *et al* 1983) and maize (Hack and Leaver 1983). Synthesis of additional polypeptides and/or disappearance of others has been observed in the mitochondria of CMS plants (see below).

4. Mitochondrial DNA and cytoplasmic male sterility (CMS)

CMS is a maternally inherited trait, which prevents viable pollen production. Widespread among higher plants, CMS is used in the production of F_1 hybrid seed and eliminates the costly procedure of hand emasculation. Male sterile cytoplasms are controlled by nuclear "restorer" genes that restore pollen fertility.

Substantial evidence has accumulated that the CMS trait, at least in certain cases, is encoded by MT DNA. In maize, three types of CMS are distinguished, designated CMS-T, CMS-C, and CMS-S according to the nuclear restorer genes that control these male sterile cytoplasms. Restriction endonuclease patterns of MT DNA from the three CMS types and normal, fertile cytoplasm display (though most DNA fragments are common to all four cytoplasms) specific bands for each of them. In contrast, CP DNA restriction patterns of the four cytoplasms were nearly identical (Pring *et al* 1979; Borck and Walbot 1982). Differing restriction patterns of MT DNA from fertile and male sterile cytoplasms have also been reported for wheat (Quetier and Vedel 1977), sorghum (Pring *et al* 1979), *Brassica* (Vedel *et al* 1982), sugarbeet (Powling 1982) and faba beans (Boutry and Briquet 1982).

Differences between fertile and male sterile cytoplasms can be revealed also *via* analysis of the polypeptides synthesized by isolated mitochondria. In maize CMS-T and CMS-C additional polypeptides of 13 kdal and 17·5 kdal, respectively, were synthesized, while polypeptides of 21 and 15·5 kdal were missing in T and C cytoplasms, respectively. In mitochondria from CMS-S, eight minor polypeptides of high molecular weight were synthesized that could not be detected in the other cytoplasms (Forde and Leaver 1980). Fertility restoration of CMS-T with nuclear restorer genes led to specific suppression of the synthesis of the 13 kdal polypeptide, while the missing 21 kdal

polypeptide in none of the restored lines had been resynthesized. No differences in polypeptide synthesis have been observed between sterile and restored CMS-C or CMS-S (Forde and Leaver 1980). In rape, too, CMS and fertile cytoplasms can be distinguished by the polypeptides synthesized in the mitochondria of the two cytoplasms. Two polypeptides of 40 and 16 kdal were synthesized solely by fertile mitochondria, whereas in CMS mitochondria two specific polypeptides of 37 and 17 kdal have been detected (Vedel *et al* 1982). The synthesis of an additional polypeptide in mitochondria of male sterile cytoplasms has also been reported for faba beans (Boutry and Briquet 1982).

Further evidence that mitochondria are involved with CMS comes from observations of the close association of disease susceptibility and the T-type of CMS in maize. CMS-T cytoplasms are preferentially susceptible to the fungus *Helminthosporium maydis*, race T (Mathews *et al* 1979). The host-specific toxin of the fungus affects only mitochondria from CMS-T, while mitochondria from normal, CMS-C, CMS-S, and chloroplasts from all cytoplasms are insensitive (Miller and Koeppe 1971). The tie between disease susceptibility and male sterility in CMS-T has been interpreted to mean that both phenomena are caused by a common, single genetic defect. This assumption is supported by the fact that mitochondria isolated from T cytoplasms which have been restored to fertility are much less sensitive to the toxin than mitochondria from nonrestored lines (Barratt and Flavell 1975). Further evidence for a close linkage between the fertility trait and toxin susceptibility comes from regeneration studies (Gengenbach *et al* 1977; 1981; Brettell *et al* 1980). Among toxin resistant plants regenerated from callus of CMS-T lines both male-fertile and CMS plants were found. Three generations of progeny testing gave no indications that the linkage between male sterility and toxin susceptibility had been broken by the selection and regeneration procedure (Gengenbach *et al* 1981).

It has been suggested that CMS-T mitochondria possess an altered intermembrane polypeptide (a possible candidate is the CMS-T specific 13 kdal polypeptide) providing a binding site for the toxin as well as for an anther-specific substance, both leading to uncoupling of oxidative phosphorylation and thereby to the two effects observed: susceptibility to disease and abnormal pollen development in the anthers. The nuclear restorer genes then would suppress the synthesis of the protein responsible for these effects (Forde and Leaver 1979). Analysis of MT DNA and chloroplast DNA from plants obtained by interspecific protoplast fusion provided evidence that the determiners of CMS are located most probably in the mitochondria (and not in the chloroplasts). Protoplasts from fertile and CMS plants of different *Nicotiana* and *Brassica* species, respectively, were fused. Those of the regenerated plants which exhibited the CMS trait, showed an MT DNA restriction pattern more close to that of the sterile protoplast donor species than to that of the fertile donor (Belliard *et al* 1978; Pelletier *et al* 1983; *cf* Gerstel 1980, for further references and discussion). No correlation between chloroplast DNA restriction patterns and the CMS trait was observed. The differences in the MT DNA pattern between the protoplast donor plants and the regenerates was interpreted to be the result of recombinational events.

5. CMS and plasmid-like MT DNA

In addition to the large, "chromosomal" MT DNA, mitochondria from a number of plant species have been shown to contain small, plasmid-like DNA. Molecules of this type have

been found in maize (Pring *et al* 1977; Kemble and Bedbrook 1980; Weissinger *et al* 1982), sorghum (Pring *et al* 1982), *Brassica* (Palmer *et al* 1983), sugarbeet (Powling 1981), faba beans and some other legumes (Negruk *et al* 1982; Boutry and Briquet 1982; Nikiforowa and Negruk 1983) and *Oenothera* (Brennicke and Blanz 1982). The best characterized plasmid-like MT DNA are those from maize CMS-S mitochondria (which are not found in normal or T and C cytoplams). These two plasmid-like DNA s designated S1 and S2 are isolated as linear molecules of 6·4 and 5·4 kb length, respectively, and resemble transposable elements as they possess terminal inverted repeats and additional regions of sequence homology (Kim *et al* 1982). Similar to adenovirus, they have a protein covalently attached to their 5′ terminus (Kemble and Thompson 1982). Recently, potential replicative forms of the S-plasmids have been identified (Kemble and Mans 1983). The nucleotide sequence of S1 and S2 has been determined (Levings and Sederoff 1983; Levings, personal communication). At least two open reading frames were detected.

It has been assumed that S1 and S2 originally have been excised from the MT genome of normal, male fertile cytoplasm to constitute the CMS-S lines, in which they are amplified as virus-like DNA (Koncz *et al* 1981). But the alternative possibility, *i.e.* exogenous origin by incorporation of (viral?) DNA, cannot be ruled out.

In Vg cytoplasms belonging to the S group and reverting spontaneously to male fertility, S2 DNA is substantially reduced. Moreover, in male sterile cytoplasms of maize, which have reverted to fertility, the S1 and S2 plasmids disappear, and changes in the MT "chromosomal" DNA do occur (Levings *et al* 1980). On the basis of these findings, S1 and S2 have directly been correlated with the spontaneous reversion to fertility occurring in CMS-S lines. Specific hybridization of the S-plasmids to unique restriction fragments characteristic for the revertants has suggested that the S-plasmids represent the male fertile element of the MT genome and that cytoplasmic reversion to male fertility is due to the integration of plasmid-like DNA into the MT "chromosomal" DNA (Levings *et al* 1980). Sequences homologous with the S-plasmids have been found integrated into the MT DNA of all maize cytoplasms (Thompson *et al* 1980). Recently, homologous sequences in the S-plasmids and the nuclear genome have been demonstrated (Kemble *et al* 1983). The results indicate that a complete copy of S1 could have been inserted into the nuclear genome of nuclear revertants. These studies also suggest that certain genomic mutations and rearrangements must have occurred. Sequences unique to S2 were not found in nuclear DNA. S1 showed homology not only to the nuclear DNA of revertants but to the nuclear DNA of all fertile and sterile maize lines tested. It was concluded, therefore, that the S-plasmids were not themselves the postulated fertility elements (Kemble *et al* 1983). Nonetheless, these data demonstrate homologous sequences between the nuclear genome, the mitochondrial genome and virus-like extrachromosomal MT DNA. The possible movement of genetic information from mitochondria to the nucleus *via* plasmid-like DNA may not be restricted to the S-plasmids of maize. There is a preliminary report that the covalently closed circular 1·94 kb plasmid found in N, T, C and S cytoplasms (Kemble and Bedbrook 1980) also shares homology with nuclear DNA sequences (Kemble *et al* 1983).

The S-plasmids are not the only small plasmid-like DNA s found in the mitochondria of maize cytoplasms. Thus, two additional plasmid-like DNA s (designated R1 and R2) have been detected in 12 male-fertile cytoplasms of Latin American maize races. These DNA species of 7·4 and 5·4 kb length, respectively, are isolated as linear molecules and appear in equimolar amounts. R1 exhibits extensive sequence homology with S1 from

maize S-cytoplasms and contains a 2.6 kb sequence unique to R1, whereas S2 and R2 are completely homologous (Weissinger *et al* 1982). It has been proposed that S1 may have arisen by a recombination between R1 and R2, and, based on comparative hybridization studies, those regions of normal MT DNA, which show hybridization with plasmid-like DNA, are more closely related to R1 and R2 than S1 and S2 (Levings *et al* 1983).

In addition to the linear double-stranded small DNA of the S-type, in the cytoplasms of male-fertile as well as all three types of CMS-maize supercoiled circular DNA of *ca* 1·94 kb has been demonstrated (Kemble and Bedbrook 1980). Furthermore, in all cytoplasms, except the T-type, another linear DNA molecule of 2·35 kb was present; and similarly as the *S*-cytoplasm C-type cytoplasms also contained two specific small, but circular DNA s of 1·57 and 1·42 kb.

No sequence homology between the circular DNA species common to all cytoplasms and the DNA-plasmid specific to C- or S-type was observed (Kemble and Bedbrook 1980). Although these data do not prove a direct relationship between the presence of certain small DNA species and CMS, there is good reason to investigate the possible involvement of these DNA s in male sterility.

Several reports demonstrate the presence of such small DNA molecules also in the mitochondria from a number of other plant species, with differences in their distribution among fertile and CMS lines. In sorghum, one of the CMS lines contained two linear plasmid like DNA s of 5·7 and 5·3 kb (Pring *et al* 1982). Substantial homology exists between them, while they also shared some homology with the S-plasmids of maize. Furthermore, four additional DNA species in the range between 1 and 4 kb were detected in sorghum mitochondria (Pring *et al* 1982). In faba beans, small DNA species have also been identified (Negruk *et al* 1982; Boutry and Briquet 1983). In addition to five small DNA s in the range of 1 to 2 kb found both in fertile and CMS lines an additional small DNA occurred only in one of the CMS lines (Boutry and Briquet 1983).

An unusual large MT DNA plasmid has been found in *Brassica campestris* and *B. napus* (Palmer *et al* 1983). It is isolated as a linear molecule, contains no detectable internal repeats and is highly conserved in nucleotide sequence. The plasmid is not detected (or greatly reduced in amount) in fertile lines, while in CMS mitochondria of certain lines it is present in moderate to highly increased amounts. No homology to chromosomal MT DNA nor to the S-plasmids of maize has been found for this *Brassica* plasmid (Palmer *et al* 1983). Supercoiled small DNA s have further been demonstrated in sugarbeet. Here, the mitochondria of CMS lines contain only one small DNA species of 1·5 kb, which is in contrast to fertile cytoplasms bearing three DNA species (including or lacking the 1·5 kb plasmid) in the range between 1·3 and 1·5 kb (Powling 1981).

The recent data on MT plasmid-like DNA indicate, but do not prove that these DNA species coexisting with the MT genome are involved in the control of the phenotypic trait CMS. Substantial evidence that at least part of them are capable of transposition and may be integrated into mitochondrial as well as nuclear chromosomal DNA makes them relevant to genetic manipulation approaching the modification of phenotypic expression in higher plants.

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The culture of manually isolated heterokaryons of *Nicotiana tabacum* and *Nicotiana rustica*

J D HAMILL, G PATNAIK*, D PENTAL and E C COCKING

Plant Genetic Manipulation Group, Department of Botany, University of Nottingham, Nottingham NG7 2RD, England

Abstract. Protoplasts derived from leaf mesophyll tissue of *Nicotiana tabacum* were fused with cell suspension-derived protoplasts of *N. rustica*. Heterokaryons were isolated using a micromanipulator and were cultured. Nuclear fusion was observed to occur in many of these heterokaryons after culturing them for a few days. Hybrid cell division was also observed. Fluorescein isothiocyanate (FITC) staining of *N. rustica* protoplasts prior to fusion did not interfere with subsequent hybrid cell division, and the FITC fluorescence was observed to persist beyond the first division stage of hybrid cells. From a total of thirty heterokaryons which were placed in a nurse culture of protoplasts of albino *Petunia hybrida*, thirteen green colonies were subsequently obtained of which six have regenerated somatic hybrid plants. Plants were characterised for their hybrid nature by analysis of vegetative and floral morphology, isoelectric focusing pattern of leaf esterases and Fraction 1 protein. All the six plants are nuclear hybrids. Chloroplast segregation appears to have occurred in these plants, with five having the Fraction 1 protein large subunit of *N. rustica*, and one having the large subunit of *N. tabacum*. Some of the plants possess sexual fertility.

Keywords. Protoplast fusion; nuclear fusion; hybrid cell division; somatic hybrid plants; tobacco.

1. Introduction

The fusion of plant protoplasts and subsequent recovery of somatic hybrid tissue and plants has attracted much interest in recent years as a method for studying interactions between plant genomes, and as a potentially useful method for crop improvement (Cocking *et al* 1981; Harms 1983). In addition to adequate cultural capability of at least one of the parents, and efficient and non-toxic methods of protoplast fusion, a selection scheme is necessary which will enable the recovery of a small number of somatic hybrid colonies from a much larger number of colonies derived from parental protoplasts. Several approaches have been successfully utilized to overcome this problem (Evans 1983).

A selection method, of general applicability is to isolate heterokaryons after fusion and culture them. Recently we described a simple method for manually isolating heterokaryons after fusion (Patnaik *et al* 1982). In this study we report the culture of manually isolated heterokaryons between leaf mesophyll-derived protoplasts of *Nicotiana tabacum* and cell suspension-derived protoplasts of *Nicotiana rustica*. The protoplasts of both these species show high division frequencies after fusogen treatment, and plant regeneration occurs on suitable media, thus necessitating a selection scheme for hybrid recovery.

* Since deceased.

2. Materials and methods

2.1 *Plant material and protoplast isolation*

Growth of, and protoplast isolation from, *Nicotiana tabacum* cv Xanthi was as described previously for wild type *N. tabacum* cv Gatersleben (Pental *et al* 1982). Seeds of *N. rustica* cv V27 (Jinks *et al* 1981) were surface-sterilized with 20% v/v Domestos (Lever Bros., UK) for 30 min, thoroughly rinsed with sterile tap water and germinated on the surface of BGS medium (Binding 1975) in the dark at 27°C. Hypocotyls, 2 cm in height, were transferred to the surface of UM medium (Uchimiya and Murashige 1974) solidified with 0·8% w/v agar (Sigma). Callus, which was formed after 3–4 weeks, was transferred to UM liquid medium (2 g callus/100 ml medium). Cell suspensions were maintained on a rotary shaker (120 rpm, 1800 lux, 24 ± 2°C) and subcultured every 7 days. New cell suspensions were initiated every 12 weeks. To release protoplasts, cells (3 days after subculturing) were incubated in an enzyme solution containing 2% w/v Rhozyme (Röhm and Haas Ltd., Philadelphia, USA), 4% w/v Meicelase P (Meiji Seika Kaisha Ltd., Tokyo, Japan) and 0·03% w/v Macerozyme R-10 (Yakult Biochemical Ltd., Nishinamiya, Japan) with 13% w/v mannitol and CPW salts (pH 5·8) (Frearson *et al* 1973). After overnight incubation at 24 ± 2°C, the enzyme solution containing cell debris and protoplasts was passed through a 64 μm nylon seive and protoplasts were purified as for *N. tabacum* mesophyll protoplasts. Protoplasts of an albino cell line of *Petunia hybrida* cv Comanche (Patnaik *et al* 1982) were isolated as for *N. rustica* suspension.

2.2 *Protoplast fusion and isolation of heterokaryons*

Fusion between protoplasts of *N. tabacum* and *N. rustica* was carried out using the high pH/Ca^{++} method (Keller and Melchers 1973). After fusion treatment, protoplasts were cultured at a density of 5×10^4/ml in 9 cm petri dishes (Sterilin Ltd. Teddington, UK) in MS-1 medium, ((Murashige and Skoog 1962) salts supplemented with 2 mg l^{-1} NAA (α-naphthaleneacetic acid), 0·5 mg l^{-1} BAP (6-benzylaminopurine), 3% w/v sucrose (pH 5·8)) with 13% w/v mannitol. Heterokaryons were identified by the presence of chloroplasts and cytoplasmic strands, and isolated at various times after fusion using a micromanipulator and bright field optics. In some cases fluorescein isothiocyanate (FITC) staining of suspension cells was carried out to confirm the heterokaryocyte nature of isolated cells (Patnaik *et al* 1982). Isolated heterokaryons were fixed and stained with carbol fuchsin to reveal the nuclei (Kao 1975).

2.3 *Culture of isolated heterokaryons and plant regeneration*

Isolated heterokaryons were cultured in 5–10 μl microdrops of MS-1 medium with 13% w/v mannitol as previously described (Patnaik *et al* 1981). Cells were removed from droplet cultures at various intervals and were either observed using UV or stained with carbol fuchsin to reveal the state of the nuclei.

For long term culture of isolated heterokaryons, they were placed in a nurse culture of protoplasts of albino *P. hybrida* which were placed in MS-1 medium with 13% w/v mannitol 3 days previously (24 ± 2°C, continuous fluorescent light of 1000 lux). Albino protoplasts of *P. hybrida* were cultured at a density of $2{\cdot}5 \times 10^4$/ml in 4 ml of liquid

medium placed over 4 ml of medium solidified with 0·6 w/v agar in 5 cm petri dishes (A/S Nunc, Kamstrup, Roskilde, Denmark).

Individual green colonies, which were detected among albino cell colonies, were removed and placed on MS-1 medium with 4·5 % w/v mannitol. After they had grown to 0·5–1 cm in diameter these colonies were individually placed on solidified MS-2 medium (MS medium with 1 mg l^{-1} BAP and 2 mg l^{-1} IAA (indole-3-acetic acid)). Shoots from regenerating calli were transferred to MS medium, solidified with 0·6 % w/v agar, and with no hormones. After 4 weeks growth, plants were transferred to the greenhouse as previously described. Portions of non-regenerating calli were transferred to MS-1 medium, solidified with 0·6 % w/v agar, for isozyme analysis. Parental callus was grown under the same conditions.

2.4 *Characterization of somatic hybrids*

Regenerating plants were characterized on the basis of their vegetative and floral morphology. Pollen viability was estimated by incubating freshly dehisced pollen in a few drops of 2 % w/v acetocarmine solution.

For biochemical characterization, isoelectric focusing of the subunits of Fraction 1 protein, (Cammerts and Jacobs 1980) was carried out with some modifications. Polyacrylamide electrophoresis of total leaf proteins was carried out using a vertical electrophoresis kit (Shandon) at 10 mA/gel for 12 hr. Isoelectric focusing of Fraction 1 protein was carried out on 1·4 mm thick horizontal gels (LKB Multiphor System, Sweden) using 1·5 % Ampholine, pH 5–8 (LKB). The maximum current was 10 mA and the final voltage was maintained at 1400 V for 6 hr. The gel was fixed in a solution containing 3·5 % w/v sulphosalicylic acid (Sigma) and 12 % w/v trichloroacetic acid (BDH). Protein was visualised by staining for 10 min at 60°C in a solution comprised of 0·15 % PAGE Blue 83 (BDH) in 25 %v/v ethanol and 8 %v/v acetic acid. Destaining was carried out in the above solution without PAGE Blue 83.

For isoelectric focusing of leaf esterases, and callus esterases, plant extracts were made as for Fraction 1 protein. Samples of 100 μg protein (in 30–40 μl), measured by the method of Lowry *et al* (1951) using lysozyme (Sigma) as standard, were applied to filter paper wicks 2 cm from the cathode on pre-cast thin gels (LKB PAG plate, pH 3·5–9, 5 % acrylamide, catholyte 1 M NaOH, anolyte 1 M H_3PO_4). Gels were run on the multiphor horizontal gel kit (LKB) for 4 hr at 4°C (maximum current of 15 mM and at a final and constant voltage of 1400V). Esterase bands were visualised following a modified procedure of Smith *et al* (1970), using 0·1 M sodium phosphate buffer, pH 7.

3. Results

Frequencies of interspecific protoplast fusion, calculated as the percentage of heterokaryons existing among intact parental protoplasts, were normally within the 1–2 % heterokaryon formation range using the described fusion procedure. Post fusion division frequencies were between 25 and 30 % for *N. tabacum* mesophyll protoplasts and between 35 and 40 % for *N. rustica* suspension protoplasts.

3.1 *Observations on nuclear behaviour and cell division of isolated heterokaryons*

The majority of heterokaryons, isolated and fixed immediately after fusion, were binucleate although some multi-nucleate heterokaryons were observed.

A time course was carried out on the division of parental protoplasts and heterokaryons. Suspension culture-derived protoplasts were observed to commence division 24 hr after fusion, although the majority divided within 2–3 days. *N. tabacum* protoplasts were observed to begin division after 48 hr with the majority of first divisions occurring within 3–5 days. Division of heterokaryons was observed 2–4 days after fusion.

In one experiment 52 heterokaryons were isolated after fusion and cultured in 5–10 μl microdrops. After 24 hr in culture, nuclear staining revealed that 34 of these heterokaryons had undergone nuclear fusion. At the time of fixation, the heterokaryons still exhibited the morphological markers of cytoplasmic strands (from the suspension parent) and chloroplasts (from the mesophyll parent). These heterokaryons did not exhibit any signs of mitosis at this stage although cell wall formation was indicated by the oval shape of the heterokaryons.

In another experiment 62 heterokaryons were isolated after fusion and cultured in 5–10 μl microdrops. In this case the suspension protoplasts had been labelled with FITC prior to fusion. Within 4 days of culture, 28 of these heterokaryons had divided at least once. Combined FITC and chlorophyll fluorescence was observed under UV light. Such double fluorescence persisted beyond the first division stage of heterokaryon-derived cells.

3.2 *Continued culture of isolated heterokaryons*

A total of 30 heterokaryons were isolated from a mass culture 1 day after fusion. None of the heterokaryons had divided at this stage but all had changed to an oval shape and were intact, indicating cell viability. These heterokaryons were placed in an albino nurse culture of protoplasts of *P. hybrida*. After 6 weeks, a total of thirteen green colonies were observed among the albino cells.

3.3 *Regeneration and plant morphology*

Regeneration of shoots was attempted as described in §2. Of the 13 colonies which were recovered, 6 regenerated shoots, which subsequently rooted and were transferred to the greenhouse (lines 5, 6, 9, 11, 12 and 13). The height of regenerated plants at flowering, was not uniform in the population of plants but was between that of *N. tabacum* and *N. rustica*. Leaf dimensions were also variable (table 1). The stem colour of all these plants was green, like the *N. tabacum* parent and unlike the recessive cream yellow stem colour of this variety of *N. rustica* (Jinks *et al* 1981).

The flowers of the six different plants were intermediate between the two parental flowers with regard to corolla shape and dimensions (figure 1, table 1). Several flower colours were also observed (table 1). Pollen viability was non-uniform in the population and was over 20% for three of the plants (table 1).

Certain abnormalities were present in these hybrids which were not present in either parent. Some leaves of all the hybrid plants exhibited occasional light green or yellow sectors. Flowers of all the hybrids occasionally exhibited abnormalities such as a split corolla or a stamen fused with the corolla. The flowers had a tendency to drop prematurely. However, a small number of seed pods were recovered in some selfing experiments. Those of line 5 showed 64% germination and those of line 6, 58% germination.

Table 1. Vegetative and floral morphology of *N. rustica* × *N. tabacum* somatic hybrid plants.

	Flowering height (1) (to nearest 10 cm)	Leaf index (2) length/width	Largest leaf dimensions length (cm)	Largest leaf dimensions Max. width (cm)	Flower colour (3)	Flower corolla Length (cm) (4)	Pollen viability (%)
N. tabacum var. Xanthi	130	1·8 ± 0·1	23	13	Neyron rose (55C)	4·1 ± 0·1	95
N. rustica var. V27	80	1·5 ± 0·1	25	17	Barium yellow (10C)	1·8 ± 0·1	100
N. rustica × *N. tabacum* somatic hybrids—							
H 5	110	2·1 ± 0·3	25	12	Neyron rose (56B)	2·9 ± 0·2	21
H 6	130	1·8 ± 0·2	29	16	Orient pink (36C)	2·8 ± 0·3	38
H 9	150	2·1 ± 0·2	27	13	Neyron rose (56A)	2·9 ± 0·1	17
H 11	160	1·8 ± 0·1	22	12	Orient pink (36A)	2·8 ± 0·2	43
H 12	140	1·9 ± 0·2	28	14	Orient pink (36 D)	2·9 ± 0·1	11
H 13	90	2·1 ± 0·3	22	11	Amber yellow (18B)	2·1 ± 0·2	4

(1) Plants grown to flower stage of compost (2) Six fully expanded leaves, from the primary stem of each plant, were measured. Leaves formed before the onset of flowering were assessed. (3) Assessed by comparison with Royal Horticultural Society, London, Colour Chart. Numbers in parenthesis represent actual colour bar reference. (4) Average of ten, normally formed flowers.

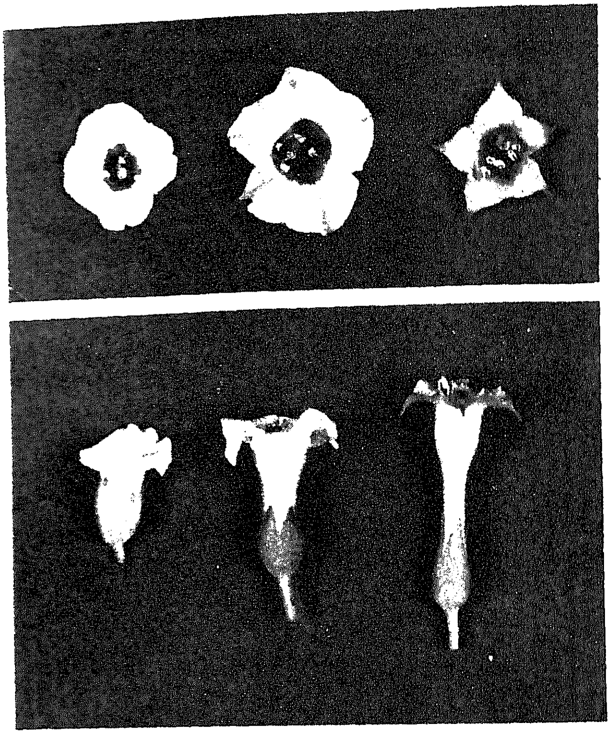

Figure 1. Floral morphology of *N. rustica* and *N. tabacum*, and somatic hybrid selected by heterokaryon isolation. Left to right.
N. rustica cv V27; somatic hybrid (line 5); *N. tabacum* cv Xanthi (× 0·65).

3.4 *Isoelectric focusing of the subunits of Fraction 1 protein*

The two species showed differences in polypeptide band profiles for both the large and small subunits of Fraction 1 protein (figure 2Ai, 2Aii). All the six hybrids had the small subunit polypeptides of both parents suggesting the presence of both nuclear genomes (figure 2Aii). Each hybrid exhibited the large subunit polypeptides of one or other parent suggesting that chloroplast segregation to have occurred. Five of the hybrid plants possessed the chloroplast Fraction 1 protein profile of *N. rustica* and one line (line 6) possessed the chloroplast Fraction 1 profile of *N. tabacum* (figure 2Ai).

3.5 *Isoelectric focusing of leaf esterases*

Both *N. tabacum* and *N. rustica* possessed leaf esterases unique to each other with respect to their isoelectric points. All six of the hybrid plants exhibited a largely additive effect of these bands (figure 2B).

3.6 *Analysis of non regenerating cell lines*

Isoelectric focusing of callus esterases (not shown) revealed only one unambiguous band present in *N. rustica* callus and not in *N. tabacum* callus. All of the seven non-

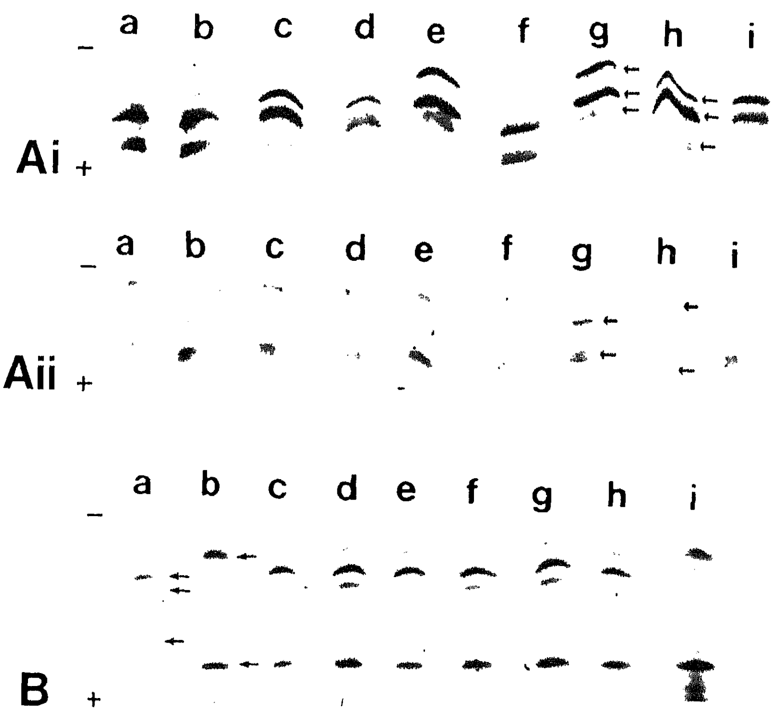

Figure 2. Biochemical analysis of *N. rustica* × *N. tabacum* somatic hybrids. (**A**) Fraction 1 protein analysis (**Ai**) Large subunit (chloroplast encoded) polypeptides. (**Aii**) Small subunit (nucleus encoded) polypeptides. (**a**) Hybrid Line 13. (**b**) Hybrid Line 12. (**c**) Hybrid Line 11. (**d**) Hybrid Line 9. (**e**) Hybrid Line 6. (**f**) Hybrid Line 5. (**g**) *N. tabacum* cv Xanthi. (**h**) *N. rustica* cv V27. (**i**) Physical mixture of *N. tabacum* and *N. rustica.* (Arrows indicate relative positions of parental bands. Uppermost bands in channels c and d of the large subunit (Ai) proved to be non reproducible and were considered artifacts). (**B**) Isoelectric focusing zymogram of leaf esterases. (**a**) *N. tabacum* cv Xanthi. (**b**) *N. rustica* cv V27. (**c**) Hybrid Line 5. (**d**) Hybrid Line 6. (**e**) Hybrid Line 9. (**f**) Hybrid Line 11. (**g**) Hybrid Line 12. (**h**) Hybrid Line 13. (**i**) Physical mix of *N. tabacum* and *N. rustica.* (Arrows indicate parental bands).

regenerating cell lines possessed this band. However, all of these lines grew on MS-1 medium as green callus, like *N. tabacum* and unlike the creamy-yellow colour of *N. rustica* under these conditions. This latter feature of *N. rustica* is attributed to the genetically recessive 'yellow' character which this plant exhibits (Hamill 1983). Thus there is some evidence that all of these seven non-regenerating cell lines possessed the nuclear genomes of both parents.

4. Discussion

4.1 *Isolation of heterokaryons*

We have demonstrated that, by using a micromanipulator and capillary pipette, coupled to a specially constructed syringe, it is possible to isolate viable heterokaryons, which in *N. rustica* and *N. tabacum* have given rise to somatic hybrid plants at a frequency of six out of the thirty heterokaryons cultured. This was facilitated by placing

the heterokaryons in a nurse culture of albino *Petunia hybrida* protoplasts demonstrating that protoplasts of a species of a different genus can act as a nurse culture for heterokaryons.

In previous studies viable heterokaryons have been successfully isolated by a variety of methods. Kao (1977) used a dilution plating technique to isolate heterokaryons of soybean and *N. glauca* in wells of Cuprak dishes. In this case cells derived from fusions were successively diluted until one heterokaryon occupied a well which was then registered. The high initial fusion frequency (39 % heterokaryon formation) and the use of a complex medium capable of supporting division of single cells were important factors in the isolation of hybrid colonies. Gleba and Hoffmann (1978) used a hand-held micropipette to isolate heterokaryons of *Arabidopsis thaliana* and *Brassica campestris* fusions, but in this case only fusion products divided and thus isolation of dividing heterokaryon-derived cells was greatly facilitated. The isolation of heterokaryons of *N. tabacum* mesophyll and *N. rustica* suspension protoplasts carried out in this study is more comparable to the isolation of *Atropa belladona* and *N. chinensis* heterokaryons (Gleba *et al* 1982). In this case, one type of parental protoplast was derived from mesophyll cells and the other from suspension cells; however, no mention was made of the fusion frequencies. In our experiments working with fusion frequencies around 1–2 %, we found that isolation with a fixed micropipette is much more convenient than isolation with a hand-held micropipette.

Hein *et al* (1983) have produced hybrid calli by isolation of heterokaryons of *N. paniculata* and *N. tabacum cnx* and *N. sylvestris* and *N. tabacum cnx* using a fixed micropipette. In this case fusion frequency was around 3 %, comparable to frequencies observed between *N. rustica* and *N. tabacum*. Isolated heterokaryons were cultured in a nurse culture of protoplasts of *N. tabacum cnx* parent which is a mutant unable to utilize nitrate as the sole source of nitrogen. Selection of the hybrid calli was accomplished by plating the entire culture on medium with nitrate as the sole source of nitrogen in which case the *N. tabacum cnx* colonies failed to grow but the hybrid calli proliferated. As the auxotrophic line of *N. tabacum cnx* was used both as a fusion parent and a nurse culture, and a selection medium was employed, the isolation of heterokaryons did not need to be stringent so as to exclude any parental *N. tabacum* protoplasts. In a similar study, Menczel *et al* (1978) used an albino, kanamycin resistant cell line of *N. sylvestris* both as a fusion parent and as a nurse culture for fusions with *N. knightiana* leaf mesophyll-derived protoplasts.

4.2 *Initial response of heterokaryons*

By isolating heterokaryons and culturing them in small droplets, we were able to follow the behaviour of nuclei at different time intervals. It seems that nuclear fusion occurs rapidly, within the first day of post-fusion culture, and before obvious signs of mitosis and cell division. These observations were not consistent with reports on nuclear fusion in animal cell heterokaryons where nuclear fusion has been reported to occur at mitosis (Harris 1974). However, nuclear fusion during interphase has been observed in plant heterokaryons by other workers. For example, in protoplasts fusion of *Pisum sativum* and *Glycine max* (Constabel *et al* 1975), and of *Daucus carota* and *Hordeum vulgare* (Dudits *et al* 1976) nuclei were observed to fuse within 24 hr of post fusion culture. Electron microscopic observation of soybean homokaryons revealed connections between interphase nuclei which may have preceded and facilitated nuclear fusion

(Fowke *et al* 1975). The situation appears analogous to a report on nuclear fusion following sexual fertilization in *Gossypium hirsutum*, where nuclear fusion occurred *via* nuclear endoplasmic reticulum coalescence and membrane bridge formation (Jensen 1964).

4.3 *Relevance of fluorescein isothiocyanate staining*

As has been shown previously (Patnaik *et al* 1982), combined FITC and chlorophyll fluorescence within the heterokaryon can be useful for confirming the heterokaryocyte nature of fusion products isolated using bright field optics. This double fluorescence may be useful for the manual isolation of heterokaryons in cases where the presence of cytoplasmic standards and chloroplasts are insufficient markers for unambiguous identification of heterokaryons. Moreover, the observation that this double fluorescence persists beyond the first division of heterokaryons may be valuable for recovering viable and dividing hybrid cells. In future, combined FITC and chlorophyll fluorescence could be used for automated cell sorting using fluorescence-activated cell sorters (Redenbaugh *et al* 1982).

4.4 *Characterization of somatic hybrids*

The six somatic hybrids of *N. tabacum* and *N. rustica* produced in this study showed variable morphology and vigour as previously reported for somatic hybrids of these species (Douglas *et al* 1981a). Some seed set was observed in four of the hybrids, and the progeny are currently being analysed for hybrid characteristics.

4.5 *Chloroplasts constitution of the hybrids*

The isoelectric focusing patterns of Fraction 1 protein given evidence for the presence of one of other parental chloroplast type being present in the hybrid plants. Five hybrids appeared to possess the plastids of *N. rustica* and one hybrid possessed the plastids of *N. tabacum*. Chloroplast sorting out is a generally observed phenomenon in somatic hybrids (Chen *et al* 1977; Scowcroft and Larkin 1981). In one previous report on somatic hybridization between *N. rustica* and *N. tabacum*, the chloroplast segregation pattern was found to be random (Iwai *et al* 1980). In this case, both the parent protoplasts used for fusion were derived from mesophyll tissue. In another report on the somatic hybridization between these two species, there appeared to be a bias for *N. rustica* chloroplasts (Douglas *et al* 1981b). In this case protoplasts of both parents were derived from cell suspensions. In our study, where *N. rustica* parental protoplasts were derived from suspension cells and *N. tabacum* parental protoplasts from mesophyll tissue, there is an apparent bias for *N. rustica* chloroplasts in the hybrids. This may suggest a competetive advantage for *N. rustica* plastids, in such hybrids, when the *N. rustica* parental cells have been grown heterotrophically. However, the existence of a hybrid plant, in this study, which possesses the chloroplasts of *N. tabacum* shows that fully differentiated plastids can be retained and the observed segregation pattern may simply be due to a random assortment as has been demonstrated for other somatic hybrids, both intra and interspecific (Chen *et al* 1977; Scowcroft and Larkin 1981).

4.6 *General applicability of manual isolation of heterokaryons for somatic hybridization studies*

A large number of dicotyledon species are amenable to protoplast isolation, culture and plant regeneration (Vasil and Vasil 1980; Lu *et al* 1982; Davey and Kumar 1983), and a number of cases have been suggested where somatic hybrids could be of practical utility (Evans *et al* 1981; Evans 1983; Ahuja *et al* 1983). Since the number of mutants available for complementation selection is low (Cocking *et al* 1981) there is clearly a need to develop methods for the isolation of heterokaryons to produce somatic hybrids.

The production of somatic hybrids, between species which are difficult or impossible to cross sexually, could be approached in two steps as was carried out in this study. The first step involves the observation of nuclear fusion and hybrid cell division using a sample of isolated heterokaryons. The use of double fluorescence, as was used in this study, may be helpful in the identification of dividing hybrid cells. Such observations may provide valuable information on the extent of nuclear fusion and reveal any incompatibility that may exist at the initial stages of culture. In the second step, the remaining heterokaryons would be cultured, either in microdrops or in a nurse culture, to assess their growth and development. Such a two step approach has been shown, in this study, to be valuable for recovering somatic hybrids of *N. tabacum* and *N. rustica*, and should be applicable to hybridization of other species which have no markers for complementation selection of hybrids in culture.

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Eukaryotic transposable elements

N K NOTANI

Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400 085, India

Abstract. Transposable elements first discovered in maize have been discovered subsequently also in bacteria, yeast, *Drosophila*, mammals etc. Structurally, eukaryotic transposable elements may be classified into two groups: ones with direct or inverse-repeat ends and the others with dAMP-rich sequence at one end. They generate direct repeats at the target site. Quite often, transposable elements are dispersed as a number of copies through the genome and at times may constitute a small but significant fraction. Their dispersal or transposition through the genome may involve excision (precise or imprecise), recombination (homologous or non-homologous) and replicative events in elements with direct or inverse repeats. dAMP-ended elements may move by reverse transcription. Maize elements can modulate gene action and yeast Tyl elements can enhance transcription. Nevertheless, evidence is not conclusive that transposable elements are involved in a major way in gene regulation and development. Structural similarities among yeast Tyl elements, *Drosophila copia* sequences and retroviral proviruses such as Rous sarcoma virus (RSV) and mouse mammary tumor virus (MMTV) suggest a formal possibility of horizontal transfers.

Keywords. Nomadic DNA; symmetrical-repeat ends; dAMP-rich end; target duplication; excision; reverse transcription; gene activation.

1. Maize controlling elements

1.1 *Historical*

Maize transposable elements, which Barbara McClintock termed as controlling elements, were first reported in 1950. These turned up quite unexpectedly in plants in which short arm of chromosome 9 had been undergoing a chromosomal type of breakage-fusion-bridge (BFB) cycle (McClintock 1950, 1951). McClintock noted that the short-arm of chromosome 9 was being broken at a specific locus, between centromere and *Wx* (*Waxy*) locus, with a high frequency. This locus was designated as *Ds* (dissociation) and the initial location was termed as the standard locus of *Ds*. Breaks at *Ds* did not occur unless another element termed *Ac* (activator) was also present in the genome. In some kernels, the *Ds* activity disappeared from the standard locus and appeared at a new location. McClintock interpreted this as a transposition. In some cases, *Ds* moved next to a gene and showed mutator activity with very few breaks occurring. This was designated a change-in-state of *Ds*. In the absence of *Ac*, *Ds* stably inhibited the gene expression with which it was conjoined but in the presence of *Ac*, mutations appeared with a high frequency. *Ac* dose itself controlled the timing of the occurrence of the mutations—higher the *Ac* dose, later the mutations occurred. Although in general the maize transposable systems were of the two-element type, it was shown (Brink and Nilan 1952; Barclay and Brink 1954) that variegated pericarp (P^{vv}) is due to the direct conjoining and modulating control of *P* gene by *Ac* which they designated as *Mp*. The reason for equating *Mp* with *Ac* was that *Mp* could induce breakage at *Ds* standard locus the same way as did *Ac*. As it turned out later, this direct control by *Ac* had implications for the origin of *Ds*. *Ac* could also move autonomously.

Although, in a number of cases the appearance of *Ds* was correlated with its disappearance from the earlier locus, this was not always so. The implication here was that whereas most of the time transposition could be occurring by excision, there were times when it may be associated with replication. In fact, the evidence from the work of Greenblatt and Brink (1962, 1963) on light variegated pericarp showed that replication may be involved in transposition. There is a pericarp type called light variegated which is deduced as having 2 *Mp*'s. At times in the background of variegated pericarp a twinned sector would appear—one sector would be completely red and the other would be light variegated, as though the red sector had lost its *Mp* and the light variegated had two *Mp*'s. Greenblatt and Brink considered the twinned sector as due to a transposition during DNA replication.

McClintock (1961) drew a parallel between the bacterial operons and controlling elements. She akinned transpositions of *Ds* to transfer of foreign operators. This hypothesis was tested by attempts to induce mutations or inactivate *Ds* conjoined to A_1 gene (coloured aleurone). In the absence of *Ac*, A_1Ds phenotype is stably colourless. Mutation or inactivation of *Ds* by known mutagenic agents was to be detected as coloured aleurone kernels. No such kernels were recovered (Chandra Mouli and Notani 1970). This hypothesis was also tested with the *spm* (suppressor-mutator) system described below. The results were negative with this system also (Neuffer 1966). The discovery of transposable elements in prokaryotes and other eukaryotes has provided some criteria and paradigms and it seems useful to treat transpositions as insertions anywhere in the genome of specific DNA elements.

Another controlling element system *spm* (suppressor-mutator) discovered by McClintock is analogous to the enhancer-inhibitor system of Peterson (1965). The expression of this system is more complex. In certain cases, with only the non-autonomous receptor element (*Rs*) at a gene locus, the gene expression is intermediate between dominant and recessive. When *spm* is present, the aleurone is colourless and shows deeply pigmented revertants (see for example Fedoroff 1983a). In other cases, like in *bz-m*13, the phenotype is normal without *spm*. In the presence of *spm*, however, the gene expression is first suppressed (inhibited) but the mutator effect brings about the gene expression.

With the discovery of transposable elements in prokaryotes and other eukaryotes, the techniques of recombinant DNA were applied to analyse the molecular structure and properties of the transposable elements. Some common denominators and 'marks' of nomadic DNA have been noted. Molecular analysis of the maize elements has of late picked up momentum.

1.2 *Molecular structure and properties of maize-transposable elements*:

So far, the molecular analysis of transposable elements has been done when conjoined with only about half-a-dozen gene loci. Most of these affect the endosperm phenotype and are located on the short arm of chromosome 9. A description of some of these is given in table 1.

To date, considerable heterogeneity has been encountered amongst the various *Ds* insertions that have been encountered. The 'neatest' picture has been reported by Sutton *et al* (1984) of *Ds* at *Adhl-F* gene coding for alcohol dehydrogenase. A 0·4 kb (405bp) insertion near the 5′ end of the gene was present in *Ds-Adhl-F* genotypes but not in the progenitor or the revertants. *Ds* is bracketed by 11 bp inverse repeats and apparently

Table 1. Description of maize genes.

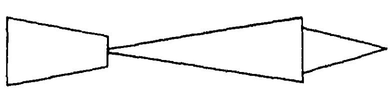
Gene	Chromosome location	Phenotype
A_1, A_2, C and R	3, 5, 9 and 10 respectively	Dominant complementary factors which condition colored aleurone
Bz	9	Recessive *bz* gives bronze-colored aleurone with A_1, A_2 C, R genotype. Codes for UDP-glucose: flavonol 3-0-glucosyl transferase
Sh_1	9	Deep-dented kernels. Codes for sucrose synthase
Wx	9	Endosperm contains amylopectin plus amylose. Codes for a particle-bound glucosyltransferase. Recessive allele *wx* contains only amylopectin.
p^{RR}	1	Red pericarp
p^{VV}	1	Veriegated pericarp
Adh_1	1	Codes for enzyme alcohol dehydrogenase

generates an 8 bp (direct) repeat at the site of insertion. The sequence is AT-rich. In two *Ac-induced* revertants, 405 bp sequence (*Ds*) is missing but the 8 bp duplication with minor changes is left behind. Thus, the excision would appear to be relatively precise. At least 30 *Ds*-like sequences were detected in maize genome by Southern hybridization.

Surprisingly, *Adhl*-specific mRNA is of similar size both for the progenitor as well as for *Ds-Adhl-F* genotypes. Since *Ds* is AT-rich like some plant introns, it is suggested that these are deleted in RNA processing. It is also suggested (Sutton *et al* 1984) that *Ds* may contain its own sites of transcription initiation and several TATA box sequences are present. The lower alcohol dehydrogenase activity is accounted for by lower mRNA levels in the *Ds*-induced mutant.

In contrast to the above results, Doring *et al* (1984b) reported a 1·3 kb insertion in a new *Ds*-induced mutation *Adhl-2F11* at the *Adhl* gene. Even more surprisingly all or nearly all of the *Ds* sequence is transcribed into mRNA which is hybridizable to a *Ds* probe. 'Mutant' mRNA in fact is even slightly larger than normal by 1·55 kb rather than 1·3 kb which is explained by a possible read-through transcription.

Sizes of the inserts determined from *Ds* transposition at Sh_1 locus are even more variable. Using a c DNA probe from *shrunken* mRNA, Burr and Burr (1981 a, b, 1982) isolated clones containing *Ds* inserts. They observed inserts of more than 20 kb in *sh-m* 5933 and *sh-m* 6233. They also observed extensive internal rearrangement. A revertant had maintained a 21–22 kb insert at the same location. Courage-Tebbe *et al* (1983) found a 30 kb insert at the *Sh* locus in *sh-m* 5933 as well as a duplication that includes part of the insert and part of the *Sh* locus on the 5′ side of the insert. The revertants still show *Ds*-mediated breakage, with intact *Sh* locus from which the insertion is removed but the duplication is retained. Doring *et al* (1984) have sequenced 4·2 kb DNA adjacent to 3′ end of *Sh* gene. This sequence consists of a duplication of the element, in which one element is inserted into the other in the following manner:



which is symbolized as *D* (*s*′ *D*′)*s*. Although the total insertion is very large, only the parts at both junctions are considered transposable. Both the 2 kb copies have ends with inverse repeats which are flanked by 8 bp direct repeats.

Sequence analysis indicates some similarities between termini of *Ds* with those of murine leukaemia and sarcoma viruses. The hexanucleotide ATGAAA is the same amongst the three. The three AAA at the end of the sequence form part of a pentanucleotide AAAGA shared with FB (fold back) element of *Drosophila* (Doring *et al* 1984).

Expression of three mutables at Sh_1 locus, *viz sh-m* 6233, *sh-m* 5933 and *sh-m* 6258, has been examined by Fedoroff *et al* (1983). *sh-m* 6233 and *sh-m* 5933 produce less than 1 % normal mRNA in endosperm tissue. But the aberrant transcripts are produced and are apparently translated into immunologically-related proteins.

Ds, at least in certain cases, appears to arise as an internal deletion of *Ac*. Thus, Fedoroff *et al* (1983) observed that *Ac* at *Wx* locus corresponds to a 4·3 kb insertion whereas 2 *Ds*'s correspond to 4·1 and 2 kb insertions. 4·1 kb *Ds* is homologous to *Ac* except for a 0·2 kb internal region. 2 kb *Ds* is homologous to the *Ac* ends. While there are many copies of *Ac*-like ends, there are fewer than 10 copies which hybridize to middle portion of *Ac*.

Recently, a report has appeared about the expression of a bronze 'mutable', *bz-ml*3, induced by the insertion of *Spm* receptor at *bz*-1 locus (Klein and Nelson 1983). Phenotypically in the absence of *Spm* (the autonomous element), the *Rs* (non-autonomous element of the system) allows full expression of *Bz*. However, Klein and Nelson note that the enzymatic product of the gene namely UDPglucose: flavonol-3-0-glucosyl transferase (UFGT), is produced in similar amounts far exceeding the enzymatic activity. The authors suggest that two polypeptides are produced from *bz-m* 13 allele.

2. Yeast (*Saccharomyces cerevisiae*)

At least two families of DNA sequences, namely transposon yeast Ty (Cameron *et al* 1979) and *sigma* (del Rey *et al* 1982), are considered to be transposable elements. Of Ty1, about 30–35 sequences are present per haploid genome. Structurally, Ty consists of a central part of a few kbp flanked by two direct repeats called *delta*. The total size of Ty1 is 5·9 kbp and that of *delta* sequence only 340 bp. Solo *delta* sequences are additionally dispersed and it is estimated that at least 100 copies of it are present per haploid genome. Both Ty and *delta* sequences display considerable heterogeneity but the two flanking *deltas* generally have identical sequences (Roeder and Fink 1983).

Substantial transcription of Ty1 occurs to yield poly (A^+) RNA. Transcripts are produced in two sizes; one 5·7 kb and the other 5 kb. Of the two, the former is the more abundant one. Transcription of Ty1 has an unexpected relationship with the mating-type locus. Thus, in a or α mating type stationary phase cells, 5–10 % of the poly(A^+) RNA is Ty1 but in a/α, it is 95 % less. Ty1, when inserted next to a gene, most often increases, but in rare cases blocks, transcription of the gene. Surprisingly, transcription of 5·7 kb transcript begins in one *delta* sequence, continues through the middle part and ends in delta sequence of the opposite end. Thus, 5′ end of the 5·7 kb transcript has been located about 95 bp in δL sequence and its end in δR at 295 bp as shown in figure 1. Intriguingly, the enhanced transcription of the gene is in the opposite orientation (Elder *et al* 1983).

A novel mechanism for transposition of Ty1 has been proposed. Because of strong

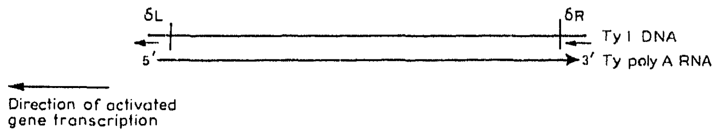


Figure 1. Ty 1 5·7 kb RNA end points and orientation of transcription of Ty RNA and the activated gene (after Elder *et al* 1983).

structural similarities of Ty1 to retroviruses, it is considered likely that reverse transcription may provide a mechanism of transposition. Of course, the presence of direct repeats should facilitate making of c DNA. In addition, Eibel *et al* (1980) pointed out that just as with retroviruses, the $tRNA_{I}^{met}$ has a homologous site just inside of the *delta* sequence which, as is known, is used as a primer for DNA synthesis (Elder *et al* 1983).

The other mechanisms of moving Ty are by gene conversion in which the sequence of one element is converted to another (opposite) type. There is also the excision, which involves *delta-delta* recombination and reintegration by recombination with a Ty element elsewhere. There is also a nonrecombinational type of transposition in which insertion is at a non-homologous site (Roeder and Fink 1983).

Sigma is a 341 bp element of which about 30 copies are dispersed per haploid genome. It is flanked by 8 bp inverse repeats and generates a 5 bp direct repeat at the target site. It appears to occur invariably 16–18 bp upstream from a t RNA locus, 25 of which have been analyzed. Of course, all t RNA loci, and there are about 300 of them, do not have *sigma* sequence upstream. Although, *sigma* has all the marks of a transposable element, no *de novo* transpositions have been detected (Brodeur *et al* 1983).

3. *Drosophila*

At least four families of transposable elements have been reported from *Drosophila*. *Copia*-like elements are characterized by long terminal direct repeats several hundred nucleotides long. These elements produce a duplication at the target site. *Copia*-like elements constitute almost 5% of the total embryonic genome (Rubin *et al* 1981). Like Ty elements of yeast, *copia*-like elements are also heavily transcribed (Finnegan *et al* 1978). Ikenaga and Saigo (1982) noted that three insertions of element 297 (a *copia*-like element) were in T-A-T-A-T-A sequence. Two of these were in the T-A-T-A boxes of H3 histone gene and one in the A + T spacer region between H1 and H3 histone genes. Thus, 297 shows site preference for insertion.

Structural and functional similarities between *copia*-like elements and retroviruses have been noted. Circular copies of *copia*-like elements with similarities to retrovirus proviruses have been observed. Kugimiya *et al* (1983) have sequenced LTR (long terminal repeats) of a *copia*-like 17·6 element which are not only similar to 297 but also to those of avian-leukosis-sarcoma virus (ALSV). 17·6 LTR's like those of ALSV, could be divided into 3 regions, *viz.* U3, R and U5 involved in transcription and reverse transcription.

Another family of transposable elements called fold back (FB) has long terminal inverse repeats with variable sequence length in between. FB also produces a target site duplication. FB elements are dispersed middle-repetitive DNA. FB induces chromosomal rearrangements. Collins and Rubin (1984) observed that out of 10 rearrangements, 6

were deletions and that sequences between two closely-linked elements were frequently deleted.

A third family of elements designated *P* elements is distinct from *copia*-like elements or FB elements. *P* appears to play an important role in hybrid dysgenesis which is manifest in two interacting strains of *Drosophila* but usually in one direction only. *P*-strains contain *P* elements which when crossed to a female strain lacking *P* element, result in hybrid dysgenesis (reviewed in Rubin 1983). *P* element has 31 bp inverse repeat.

A fourth family of transposable elements called *F* has been reported by Dawid *et al* (1983). About 60 copies of *F* occur at chromocentric sites and 25 at euchromatic sites. *F* elements are about 4·7 kb in length, do not have LTR (direct or inverse), induce a duplication of 8–13 nucleotides at the target site and at one end they carry a stretch of 12–30 adenylate residues. They appear to have some similarities with *Alu* sequences and processed pseudogenes of mammals.

4. Miscellaneous

4.1 *Alu* and *KpnI sequences*

Related dispersed repeats in eukaryotes are suggested to be mobile elements. The *Alu* family of sequences constitutes about 5 % of the mammalian genome. *Alu* sequences do not have symmetrical ends. So they are considered to transpose by a different mechanism. Their 3′ ends have α-AMP rich sequence that are different from their 5′ end sequence. But *Alu* sequences are flanked by a bigger, 7–20 bp, duplication at the target site. Another family of sequences not related to *Alu* is *Kpn*I which may nevertheless have properties of transposition etc in common with *Alu* sequences. He La cells contain $3\text{–}4{\cdot}5 \times 10^4$ *Kpn*I sequences. *Kpn*I sequences appear to transcribe well in certain lines. Some discrete sized RNA in the cytoplasm are poly(A)-terminated. DiGiovanni *et al* (1983) have presented evidence which suggests that human *Kpn*I poly(A)-terminated RNA is reverse transcribed and integrated into the genome.

4.2 *Viroids*

Kiefer *et al* (1983) suggest that viroids may have originated from transposable elements or retroviral proviruses by deletion of internal sequences of the virus or transposable element. Viroids are single-stranded circular RNA molecules which are not wrapped in a protein coat. They are nevertheless infectious and cause diseases in plants *e.g.* potato spindle tuber viroid. The similarities with transposable elements include the presence of inverted repeats ending in nucleotides UG and CA and flanking direct repeats (although imperfect). Another common feature includes long uninterrupted purine sequences in all 5 viroids which can be the primer for reverse transcriptase.

4.3 *Transposable DNA in maize mitochondria*

S-1 and S-2 DNAS, which although are extracted with the maize mitochondrial DNA but are nevertheless distinct from it, appears to condition S cytoplasmic male sterility. In fertile revertants, S-1 and S-2 DNAS are no longer seen. Fertility can also be restored by nuclear gene *Rf*3 located on chromosome 2. S-1 and S-2 DNAS are 6·4 and 5·4 kb long and are isolated as double-stranded linear DNA. These molecules have 0·2 kb terminal inverse

repeats. Apart from a free phase, S-1 and S-2 sequences are found integrated in all maize mitochondrial DNAs. Levings and Sederoff (1983) have sequenced 5452 bp of S-2 DNA. It is terminated by an exact 208 bp inverse repeat. They found two open-reading frames: a 3294 bp (1098 amino acids) and a 1017 bp one (339 amino acids). As with adenovirus and *B. subtilis* phage DNA s, Kemble and Thompson (1982) have found that the 5′ termini of S-1 and S-2 are covalently linked to proteins which are suggested to be involved in primary replication. It is not known if the open reading frames are coding for a transposase function or for the covalently-linked proteins.

5. Discussion

Eukaryotic transposable elements may fall into two structural groups: One with symmetrical ends, *i.e.* direct or inverse repeats and the other with *d*-AMP rich sequence at one end. The movement of the former may involve excision (precise or imprecise), recombination (homologous or nonhomologous), replicative events etc. The second group of elements may move through reverse transcription and may include such elements as *Kpn* I and *Alu* sequences, intronless genes, some U1 RNA pseudogenes and *F* element of *Drosophila*. Rare as these events are, they nevertheless indicate a complex traffic in DNA metabolism.

When first discovered in maize, the transposable elements were called controlling elements by McClintock. The implication was that these elements have a natural role in regulation and development. As of now, the evidence does not seem to be conclusive on this point; although a role for transposable elements in determining yeast mating types and variable surface glycoprotein genes of Trypanosomes seem well established. T-segment of Ti plasmid of *Agrobacterium tumefaciens* which following infection is transferred to dicotyledonous plant genome has the 'earmarks' of transposable element and which induces gene expression that is tumorigenic for the plant but which also facilitates the bacterial growth. There is another suggestion that repeated DNA sequences are simply selfish DNA meaning that they are parasitic and have no specific function (Doolittle and Sapienza 1980; Orgel and Crick 1980). One thing, however, seems apparent that these elements are capable of generating lot of diversity.

In terms of increasing gene expression, Ty1 of yeast is enigmatic. Not only is the expression of certain genes increased but the transcription of these is in the opposite direction of that of Ty itself. Somehow, this type of behaviour is reminiscent of the action of enhancers discovered recently which seem to increase expression of antibody genes both from upstream or downstream direction.

The structural similarities amongst Ty1, *copia* and retroviral proviruses of rous sarcoma virus (RSV) and mouse mammary tumor virus (MMTV) are striking (see for example Varmus 1983) and these suggest a formal possibility for the horizontal transfer of these elements. Proviruses share with transposable elements other properties like insertion mutagenesis, excision by recombination and activation of the expression of neighbouring genes.

Of all the transposable elements only the maize systems appear to be of the two element type. The frequency of mutation or breakage is determined by the state of the non-autonomous locus (*e.g. Ds*) and the timing by the *Ac* dose. A *Ds* is inferred to arise from *Ac* by internal deletion. It is not known whether this abolishes a transposase function. The concept of *Ac* dose has become a little ambiguous since at that time only

one copy was expected to be present per haploid genome and now it is known that number of *Ac* copies in certain stocks may be as high as 10. The vector, mutator or enhanced expression properties of transposable elements are utilizable in principle in biotechnology. Some of these have been discussed in a recent report (Notani and Bhatia 1984).

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Feulgen microspectrophotometric estimation of nuclear DNA of species and varieties of three different genera of Marantaceae

A K SHARMA and SANDIP MUKHOPADHYAY

Centre of Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India

Abstract. Karyological analysis including determination of somatic chromosome number, total chromosome length, volume and karyotype formula and *in situ* estimation of 4C-nuclear DNA amount were carried out on 14 different species and varieties of the genera *Calathea*, *Maranta* and *Stromanthe*. The 4C nuclear DNA amount was estimated through Feulgen microspectrophotometry following single wavelength method and expressed in arbitrary units of relative absorbances. The variation in 4C DNA amounts between the species of *Calathea* was not distinct but in two species of *Maranta*, a notable variation in nuclear DNA amount was recorded. In addition, the amount of nuclear DNA did not show direct correlation with the total chromosome length and volume. The absence of wide difference in nuclear DNA content at an interspecific level might have some adaptive value.

Keywords. Feulgen microspectrophotometric estimation; nuclear DNA; Marantaceae.

1. Introduction

The importance of the study of DNA content is being increasingly realized in different facets of chromosome research (Evans *et al* 1972; Price 1976; Sharma 1983). The data collected from different centres indicate the occurrence of both phylogenetic and ontogenetic increases in DNA content during evolution (Nagl 1976, 1977). Such phylogenetic increase has not been a universal feature and cases have been recorded where the evolutionary progress has not been associated with the increase in DNA content (vide Price 1976). A compensatory mechanism for the lack of phylogenetic increase has also been claimed to be due to an increase in DNA content during ontogeny.

Extensive studies have been carried out in later years on *in situ* quantitation of DNA at interspecific and at intergeneric levels (Chooi 1971; Paroda and Rees 1971; El-Lakany 1972; Martini and Brunori 1973; Smith and Bennett 1975; Stucky and Jackson 1975; Teoh and Rees 1976; Iyenger and Sen 1978; Nagato *et al* 1981; Ohri *et al* 1981; Resslar *et al* 1981). Data in this aspect have been rather contradictory, though within a genus the amount of DNA indicates a wide range (Furuta *et al* 1975; Narayan and Rees 1976; Bennett *et al* 1977; Price *et al* 1980; Raina and Rees 1983; Seal 1983). Simultaneously, there are records where different species of the same genus do not necessarily show a heavy difference in the content of DNA (Gupta and Rees 1975; Resslar *et al* 1981). However, in general, the large amounts of DNA often noted in several genera have been attributed to the presence of reiterated sequences or more precisely, repeated sequences. The function of such repeated sequences has been differently interpreted by various authors (Jacq *et al* 1977; Strobel *et al* 1979; Brown and Dover 1979; Doolittle and Sapienza 1980; Orgel *et al* 1980; Zuckerkandel 1980; David *et al* 1981). Their role in the control of non-specific functions as well as some biophysical parameters (generation time, cell size, cell volume, mitotic index) has been reiterated.

Massive changes in nuclear DNA amount in related species which maintain a consistency, specially related to repetitive and non-repetitive sequences, have been recorded by several authors (Bullen and Rees 1972; Ayonoadu 1974; Bennett *et al* 1977; Rees and Narayan 1977; Rees *et al* 1979). In view of this fact, the role of natural selection in maintaining a particular DNA fraction having specific range of tolerance has been suggested (Hutchinson *et al* 1980). It has also been indicated that, in order to ascertain precisely the importance of DNA in species diversification and phylogeny, a full understanding of the intraspecific differences, if any, is required, as recorded in certain species (Sharma 1983).

Several factors have been shown to affect the variation in DNA amount of which inbreeding and outbreeding are important ones (Rees and Jones 1967; Gupta and Rees 1975; Hutchinson *et al* 1979). The correlation of variable DNA with chromosome volume, chromosome size as well as other parameters of the cell has been explored by several authors (Evans *et al* 1972; Bryant 1973; Price *et al* 1973; Nagl 1974; Edwards and Endrizzi 1975; Murray 1975; Gupta 1976; Sharma and Chattopadhyay 1983).

In this subcontinent, the species of *Maranta*, *Calathea* and *Stromanthe* are widely cultivated because of the ornamental value of the foliage leaving aside the arrowroot yielding species of *Maranta*. Cytological studies, so far carried out in this genus earlier from this centre, have indicated the haploid number to be mostly 12 or 13 with a few aneuploids with multiples. Most of the species of *Calathea*, however, are diploids. *Maranta* and *Stromanthe* show high chromosome numbers. A comprehensive analysis of different facets of chromosome research on this group has been undertaken in this laboratory, of which *in situ* estimation of nuclear DNA forms an integral part. The present study principally deals with the DNA estimation on 11 different species and varieties of *Calathea* and three species of *Maranta* and *Stromanthe*. All these species propagate vegetatively and as such the factors of in- and out-breeding are eliminated. It was desired to find out the extent to which the values of DNA are indicative of the relationship and to what extent they have a selective advantage. Moreover, their correlation with the volume, size and other parameters of chromosome has also been analysed.

2. Materials and analysis of results

2.1 *Materials*

In situ estimation of 4C nuclear DNA amount has been carried out on 14 different species and varieties of three different genera *Calathea* Mey., *Maranta* Plum ex L. and *Stromanthe* Sond., under the family Marantaceae Petersen. These plants were: (i) *Calathea lietzei* E. Morr., (ii) *C. undulata* Regel., (iii) *C. princeps* Regel., (iv) *C. picturata* C. Koch. & Linden. var. *vandenheckii*, (v) *C. picturata* C. Koch. & Linden. var. *argentea*, (vi) *C. zebrina* Lindl., (vii) *C. clossoni*, (viii) *C. bachemiana* E. Morr., (ix) *C. kegeliana*, (x) *C. insignis* Petersen, (xi) *C. ornata* Koern. var. *roseo-lineata*, (xii) *Maranta arundinacea* L. var. *variegatum*, (xiii) *M. bicolor* Ker-Gawl. and (xiv) *Stromanthe sanguinea* Sond.

For somatic chromosome study, pretreatment of the root tips in aqueous solution of paradichlorobenzene (1/4th saturation) at 18–20°C for 5 hr was followed by overnight fixation in acetic acid-ethanol mixture (1:3); cold hydrolysis in 5N HCl at 20°C for

15 min to remove cytoplasmic contents and staining in 2% acetic-orcein staining solution for 4 hr.

Individual chromosome volume was calculated from the formula from metaphase plates as:

$$\text{Chromosome volume } (V) = \pi r^2 h,$$

where r = radius of the chromosome = breadth/2 and h = length of the whole chromosome. The total chromosome volume was then expressed by adding the volumes of all the chromosomes of a complement.

For estimation of 4C nuclear DNA amount, root tips were fixed in glacial acetic acid and ethanol mixture (1:3) for 2 hr at room temperature, hydrolysed in 1N. HCl solution at 60°C for 10 min; washed in distilled water; kept in 45% acetic acid for 5 min and stained in Feulgen staining solution (Schiff's reagent) for 2 hr, followed by squashing in 45% acetic acid.

Cytophotometric analysis was done in a reichert Zetopan microspectrophotometer following single wavelength (550 nm) method (Sharma and Sharma 1980) from 4C nuclei at metaphase.

The 4C nuclear DNA amount was measured on the basis of optical density in terms of arbitrary units of relative absorbances.

3. Results

3.1 *Chromosome characteristics*

The somatic chromosomes of 14 different species and varieties of the genera *Calathea*, *Maranta* and *Stromanthe* were analysed. The observed diploid chromosome numbers of these species were $2n$ = 24, 26, 28, 44, 48 and 52. The karyotype formulae of all these plants clearly revealed the role of numerical and structural alterations of both nucleolar and centromeric chromosomes in speciation of these genera (figure 1 and table 1).

The total chromosome length of the species of *Calathea* ranged from 25·86–44·84 μm (table 1). In the two species of *Maranta* the total chromosome length varied quite significantly in spite of a little difference in their somatic chromosome number (table 1). In *Stromanthe sanguinea* the value was 56·78 μm, which was more or less similar to that of *M. bicolor* (table 1).

Regarding the total chromosome volume, a remarkable variation was noted amongst the species of *Calathea*, where the values ranged from 5·58–27·27 cu. μm. In *M.*

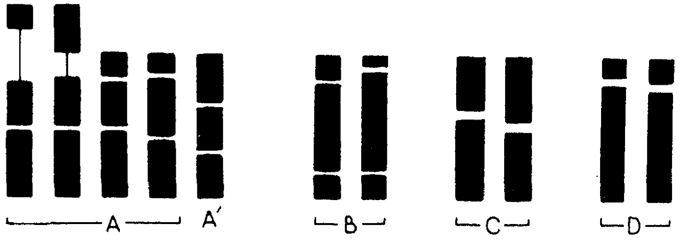

Figure 1. Diagrammatic representation of common chromosome types present in species of *Calathea*, *Maranta* and *Stromanthe*.

Table 1. Relative amount of nuclear DNA in different species and varieties of *Calathea*, *Maranta* and *Stromanthe* along with the values of other cytological parameters.

Name of the species	Somatic chromosome number (2*n*)	Karyotype formula	Total chromosome		Nuclear DNA amount (in arbitrary unit)
			length (μm)	volume (cu. μm)	
Calathea lietzei E. Morr.	24	$A_4C_{18}D_2$	37·76 ± 0·06	23·36 ± 0·10	0·1344 ± 0·0017
C. undulata Regel.	24	A_4C_{20}	25·86 ± 0·08	5·58 ± 0·02	0·1298 ± 0·0026
C. princeps Regel.	24	$A_2B_2C_{20}$	33·12 ± 0·08	10·83 ± 0·03	0·1052 ± 0·0013
C. picturata C. Koch. & Linden. var.	26	$A_2B_2C_{20}D_2$	36·96 ± 0·10	14·62 ± 0·04	0·1250 ± 0·0021
vandenheckii	26	D_2			
C. picturata C. Koch. & Linden. var. *argentea*	26	$A_4B_2C_{20}$	37·44 ± 0·06	12·90 ± 0·04	0·1364 ± 0·0027
C. zebrina Lindl.	26	$A_2B_2C_{20}D_2$	32·32 ± 0·05	12·84 ± 0·03	0·1202 ± 0·0029
C. clossoni	26	$A_2A'_2C_{22}$	44·84 ± 0·06	27·27 ± 0·05	0·1280 ± 0·0032
C. bachemiana E. Morr.	26	$A_4B_2C_{16}D_4$	44·44 ± 0·07	11·01 ± 0·03	0·1286 ± 0·0026
C. kegeliana	28	$A_4B_2C_{16}D_6$	42·84 ± 0·06	11·70 ± 0·03	0·1750 ± 0·0018
C. insignis Petersen	28	$A_2B_2C_{24}$	35·50 ± 0·05	9·82 ± 0·03	0·1332 ± 0·0019
C. ornata Koern. var. *roseo-lineata*	28	$A_4C_{22}D_2$	34·86 ± 0·06	11·72 ± 0·04	0·1480 ± 0·0031
Maranta arundinacea L. var. *variegatum*	48	$A_6C_{40}D_2$	65·18 ± 0·05	17·41 ± 0·02	0·1262 ± 0·0026
M. bicolor Ker-Gawl.	52	$A_6B_2C_{44}$	56·08 ± 0·03	12·56 ± 0·01	0·1734 ± 0·0029
Stromanthe sanguinea Sond.	44	$B_8C_{24}D_{12}$	56·78 ± 0·05	12·99 ± 0·02	0·2254 ± 0·0028

arundinacea, *M. bicolor* and *S. sanguinea* the values were 17·41, 12·56 and 12·99 cu. μm respectively (table 1 and figure 3).

3.2 *Amount of* DNA

The DNA values along with the other cytological parameters have been expressed in table 1 and figures 2 and 3.

In species of *Calathea*, the 4C DNA values did not differ significantly ranging from 0·1052 to 0·1750 units, in spite of the major variations in their chromosome volume. For example, in *C. undulata* and *C. clossoni* the DNA values were 0·1298 and 0·1280 units whereas the chromosome volumes were 5·58 and 27·27 cu. μm respectively (table 1 and figure 3). Furthermore, in *C. lietzei* the volume was greater than that of *C. kegeliana*, though the DNA value of the latter (0·1750 unit) was more than that of the former (0·1344 unit) (table 1 and figure 3).

Amongst the two species of *Maranta*, the DNA values were directly proportional to their somatic chromosome number and inversely proportional to their chromosome volume. However, in *M. arundinacea* and *M. bicolor* the 4C DNA values were 0·1262 and 0·1734 unit respectively (table 1 and figures 2 and 3). In single species of *Stromanthe*, the DNA amount was maximum (0·2254 unit), though its total chromosome volume was quite less (table 1 and figures 2 and 3).

Apart from these, no direct relationship could be obtained between the chromosome volume and amount of DNA in the different species and varieties studied, so far.

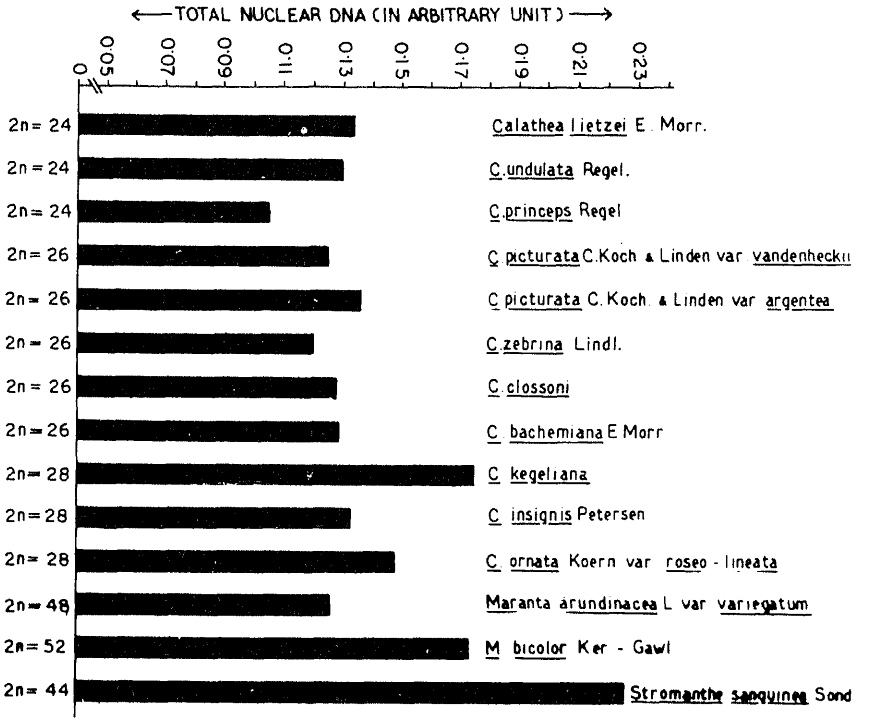

Figure 2. Histogram plate showing 4C nuclear DNA amount in different species of *Calathea*, *Maranta* and *Stromanthe*.

4. Discussion

4.1 *Chromosome number and chromosome size*

The chromosome numbers of different species of *Calathea* studied show three species with $2n = 24$, five with $2n = 26$ and three with $2n = 28$ chromosomes. As such, the difference in chromosome number is not wide showing $n = 13$ chromosomes in most of the species. Previous reports have, however, reported $n = 11$ chromosomes in several species. There is the possibility that these numbers are mostly derivatives of each other. On the basis of available evidences, a comparatively primitive member appears to have $n = 4$ chromosomes (Sharma and Bhattacharyya 1958). In that event, duplication of chromosomes followed by structural changes might have been an important factor in evolution. This is also brought out by the karyotype formula which shows minute differences from one another, even though following a consistent pattern in gross morphological details.

However, of the different species studied, *C. clossoni*, *C. bachemiana* and two varieties of *C. picturata* have rather long chromosomes as compared to the rest. This increase in size has affected the total chromosome complement. Leaving aside the difference in size of the chromosome in these species, the overall nature of the chromosome complement indicates the homogeneity of the assemblage.

4.2 *Chromosome length, karyotype and the nuclear DNA amount*

As far as the length of the chromosomes is concerned, which has been analysed from a

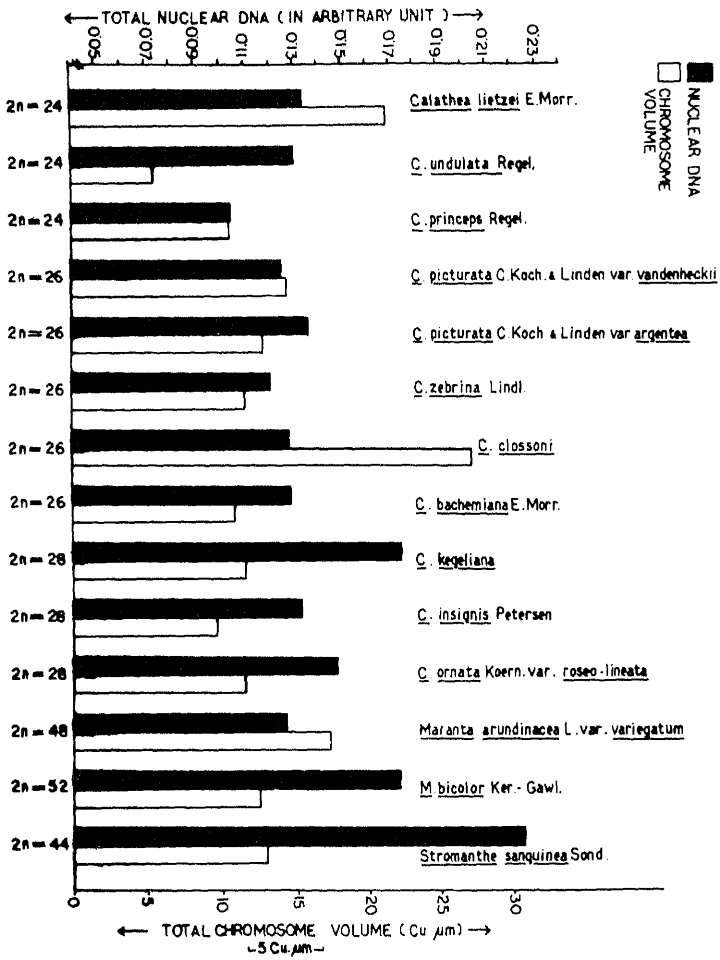

Figure 3. Comparative histogram representing 4C nuclear DNA amount and total chromosome volume in species of *Calathea*, *Maranta* and *Stromanthe*.

large number of metaphase plates following a common method of treatment, the total chromosome length ranges between 25·86 μm (in *C. undulata*) and 44·84 μm (in *C. clossoni*). This is quite remarkable as the range in chromosome number is between $2n = 24$ to 28. From the karyotype analysis, as found in different papers, it has been noted that the increase in length has affected almost all chromosomes of the complement. Such a wide difference in chromosome length may either be attributed to the presence of additional gene sequences or the despiralized nature of the chromosome thread, even making allowances for four more chromosomes.

The study of DNA content, on the other hand, reveals a rather consistent picture, excepting in *C. kegeliana*. In *C. undulata* the nuclear DNA amount is 0·1298 in arbitrary units with a chromosome length of 25·86 μm. On the other hand, more or less similar DNA value (*i.e.* 0·1286 unit) is found in *C. bachemiana*, where the chromosomes are quite large with a total chromosome length of 44·44 μm. Similarly, *C. clossoni*, with rather

long chromosomes, has more or less equal amount of DNA (0·1280 unit), though the total chromosome length is 44·84 μm. In *C. picturata* var *argentea* the total chromosome length is 37·44 μm. This is less than those of *C. bachemiana* and *C. clossoni* having the same chromosome number but the DNA value is slightly more than the latter two. These facts clearly indicate that in the genus *Calathea*, the apparent increase in chromosome length has not necessarily been associated with increase in the amount of DNA. The comparatively high DNA value has, however, been recorded in three species of *Calathea* with $2n = 28$ chromosomes. But in *C. lietzei* ($2n = 24$) with a total chromosome length of 37·76 μm the amount of nuclear DNA is more or less equal to that of *C. insignis* with $2n = 28$ chromosomes.

All these facts clearly suggest that in the genus *Calathea*, the total chromosome length is not directly correlated with the amount of DNA present in the complement. In the absence of any such correlation, the difference in length may be attributed to the differential condensation and spiralization of the chromosome arm. To what extent such processes are associated with the expression of specific genetic characters, is yet to be ascertained. However, the genetic control of the factors, responsible for difference in chromosome length, is exhibited by their constancy in all the plates studied in successive sets of experiments. Such consistency of chromosome size in different species possibly indicates an adaptive value, correlated with the physiological characteristics of the individuals.

The two species of *Maranta*, *M. arundinacea* and *M. bicolor* having $2n = 48$ and 52 chromosomes, exhibit total chromosome lengths as 65·18 and 56·08 μm respectively. The total amount of nuclear DNA in *M. bicolor* is 0·1734 in arbitrary unit as compared to 0·1262 in *M. arundinacea.* This difference in nuclear DNA may be attributed to the increase in chromosome number in *M. bicolor*. As far as the total chromosome length is concerned, like *Calathea*, no positive correlation could be established between the chromosome length and amount of DNA. In the genus *Stromanthe*, of which only one species is studied with $2n = 44$ chromosomes, the total chromosome length comes very near to that of *M. bicolor* but the nuclear DNA amount is rather high being 0·2254 unit. This fact confirms once more the absence of any correlation between these two parameters.

4.3 *Nuclear DNA amount in relation to total chromosome volume*

The data on total chromosome volume in different species of *Calathea* shows a wide variation ranging from 5·58 cu. μm in *C. undulata* to 27·27 cu. μm in *C. clossoni*. Such a difference in total chromosome volume does not bear any correlation with the chromosome number. For example, the two species, *viz C. clossoni* and *C. bachemiana* with $2n = 26$ chromosomes, have total chromosome volume of 27·27 and 11·01 cu. μm respectively. On the other hand, the total chromosome volumes of *C. picturata* var. *argentea* and *C. zebrina* having $2n = 26$ are more or less identical. Amongst the 24 chromosome complements, remarkable difference in total chromosome volume has been recorded between *C. undulata* and *C. lietzei*, variation being 5·58 and 23·36 cu. μm respectively. Simultaneously, it is to be reiterated that amongst individuals of the same species, the chromosome volumes always remain the same indicating constancy and possible genetic control.

The amount of DNA does not also show any correlation with the total chromosome volume. The chromosome volumes in *C. undulata* and *C. clossoni* are 5·58 and

27·27 cu. μm, though the DNA amounts are more or less similar being 0·1298 and 0·1280 units respectively. Similarly, in *C. picturata* var *argentea* and *C. lietzei* the volumes represented are 12·9 and 23·36 cu. μm, whereas the amounts of DNA are 0·1364 and 0·1344 units respectively in these two species.

The volume of the chromosome, however, in an eukaryotic system is determined not only by DNA but its components, that is, basic and non-basic proteins as well. All these components enter into the composition of the chromosome structure. For the sake of argument, the role of multistrandedness of DNA may also be suggested; but the absence of any direct correlation between the chromosome volume or width with the amount of DNA, rules out the existence of any such lateral multiplication. In view of the exclusion of lateral multiplication as a contributing factor to the width of chromosome, otherwise a constant feature, the importance of protein components, playing a crucial role in the determination of chromosome volume, is indicated. A detailed cytochemical analysis, utilizing specific enzymes and interferometry for protein estimation, may provide positive clues in this direction. Investigations on this aspect are in progress. On the basis of available evidences, it may, however, be claimed that the amount of DNA in species of *Calathea* is not positively correlated with the total chromosome volume. The genetic control of the chromosome volume to which protein components play a very important role is manifested in their consistency.

Two species of *Maranta*, *M. arundinacea* and *M. bicolor* with $2n = 48$ and 52 chromosomes, show chromosome volumes 17·41 and 12·56 cu. μm, whereas the total amount of DNA is 0·1262 and 0·1734 units respectively. Such inverse correlation has also been recorded in total chromosome length as well. The inference regarding chromosome volume and the amount of DNA, as deduced from the data of *Calathea*, holds good in this genus too. In *S. sanguinea*, as one species was studied, no comparative analysis could be carried out.

4.4 *Amount of DNA and species diversity*

Consistency has been recorded in the amount of DNA in metaphase plates of the species studied. However, in *Calathea*, the total amount of DNA in arbitrary units does not show a marked difference ranging between 0·1052 in *C. princeps* and 0·1480 in *C. ornata*, excepting in *C. kegeliana* where the amount is rather high being 0·1750 unit. The three species with $2n = 28$ chromosomes show slightly higher DNA value as compared to the rest with $2n = 24$ and 26 chromosomes, excepting in *C. lietzei*. In the latter, even though the chromosome number is rather low being $2n = 24$, the chromosomes are fairly large and the nuclear DNA amount is higher than that of *C. insignis* with $2n = 28$ chromosomes. Such comparatively high amount of DNA in species with low chromosome number, is quite remarkable. To what extent, this amount is due to repeated sequences is not fully known. However, the constancy in the amount of DNA in each species as well as its consistent difference from other taxa clearly suggest that the amount of DNA may be considered as an identifying character in this genus. Simultaneously, the absence of any wide difference at an interspecific level is suggestive of their adaptive value. Possibly, the range in DNA amount between species of *Calathea* has a selective advantage, acquired through a period of evolution. The extent to which the differences so far noted are be attributed to repeated or other sequences, is not yet fully resolved. The phylogenetic increase or decrease in DNA amount, in species of *Calathea*, could not be fully ascertained due to their low range of difference. In two

species of *Maranta*, the difference in amount of DNA may, however, be due to the difference in the number of chromosomes.

The different species studied in the present context have been subjected to *in situ* estimation of DNA and analysis of size, volume and morphology of chromosomes. It is indicated that duplication of chromosomes as well as minute structural changes might have been important factors in evolution. The total chromosome length of the species differs from each other but maintains a constancy in different individuals of the same species. The difference in length is not correlated with the amount of nuclear DNA. In the absence of correlation with the amount of DNA, this difference in total chromosome length has been attributed to differential condensation and spiralisation of the chromosome thread. It is suggested that such difference in chromosome size might have some adaptive value, associated with some physiological features of the species.

As far as the total chromosome volume is concerned, a remarkable variation has been noted between different species. This difference in total chromosome volume does not show any direct relationship with the chromosome number. For example, the total chromosome volumes of *C. lietzei* and *C. undulata* are 23·36 and 5·58 cu. μm respectively, though both have $2n = 24$ chromosomes. The chromosome volume of different individuals of the same species shows an uniformity, suggesting genetic control. The nuclear DNA amount is not also proportional to the total chromosome volume. In the absence of any correlation between the chromosome volume and the nuclear DNA content, it is inferred that the components, other than DNA, are possibly responsible for the difference in chromosome volume. Investigations on the role of proteins are in progress.

In *Calathea*, the total amount of nuclear DNA does not show wide difference between different species but a consistency in the minute difference of the amount of DNA in all the species is indicated excepting a few where the content is remarkably high. In order to account for such differences, the possible role of repetitive or other sequences is yet to be explored. In addition, constancy in the amount of nuclear DNA in each species and the consistent difference at an interspecific level indicate the genetic control and selective advantage of the amount of nuclear DNA.

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Nyctanthes is a member of the Oleaceae

RUTH KIEW and PIETER BAAS*

Department of Biology, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia

*Rijksherbarium, Schelpenkade 6, Leiden, The Netherlands

Abstract. The attributes of *Nyctanthes* (habit, floral morphology, fruit and seed, stem and leaf anatomy, flower vasculature, embryology, pollen, chromosome number and phytochemistry) are reviewed and found compatible with accommodation of the genus in the Oleaceae, tribe Jasmineae. The treatment of *Nyctanthes* in Verbenaceae or as a separate family does not reflect its true affinities.

Keywords. *Nyctanthes*; Oleaceae; Verbenaceae; morphology; anatomy; embryology; karyology; phytochemistry.

1. Introduction

Since Airy Shaw (1952) removed *Nyctanthes* from the Oleaceae to subfamily Nyctanthoideae in the Verbenaceae, information has accumulated on its anatomy, embryology and phytochemistry which enables it to be compared with the range encountered both in the Oleaceae and the Verbenaceae. While most authors consider the characters of *Nyctanthes* compatible with those of the Oleaceae, Kundu and De (1968) suggest that a new family should be erected for the two species of *Nyctanthes*, *N. arbor-tristis* L. and *N. aculeata* Craib.

The following is a summary of the information now available for comparing *Nyctanthes* with other members of the Oleaceae, with new data on the anatomy of *N. arbor-tristis*.

2. Comparison of *Nyctanthes* with other members of the Oleaceae

2.1 *Habit*

As mentioned by Airy Shaw (1952), *Nyctanthes* does not "look" oleaceous. It is a coarse shrub with quadrangular stems and scabrid, dentate leaves; characters which are common in Verbenaceae. However, serrate or dentate leaves are also found in species of *Olea*, *Osmanthus* and *Myxopyrum*. A quadrangular stem is typical of *Myxopyrum* and is also found in a few Malesian species of *Jasminum*, such as *J. insigne* Bl. The thorns on the stem of *N. aculeata*, however, have no parallel in any genus of the Oleaceae.

The roughness of the upper surface of the leaf is so pronounced that leaves of *N. arbor-tristis* are used as sandpaper in parts of India where it is indigenous. However, in the Oleaceae *Menodora scabra* A. Gray has leaves which are equally scabrid.

2.2 *Floral morphology*

The flower of *Nyctanthes* has between 4 and 9 contorted lobes. *Jasminum*, *Menodora* and *Schrebera* all have more than 4 lobes (usually between 4 and 8) but these are imbricate. Apart from the aestivation, the flower is typically oleaceous: it is regular with two epipetalous stamens which alternate with the two locules of the ovary. This combination of characters is not found in the Verbenaceae.

Patel (1960) noted that *Nyctanthes* is heterostylous, as is commonly the case in *Jasminum* species. *Schrebera* is also heterostylous. Patel also noted that occasional flowers of both *Jasminum* and *Nyctanthes* have three stamens and trilocular ovaries.

2.3 *Fruit and seed*

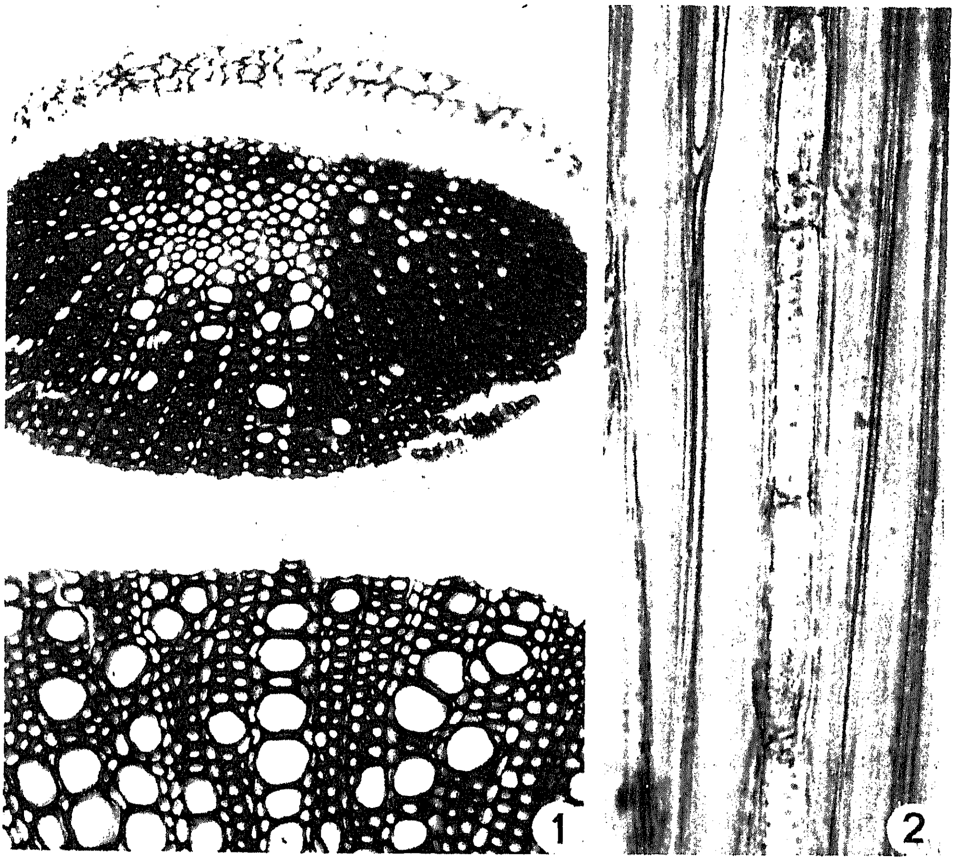
The fruit of *Nyctanthes* is a dry chartaceous capsule which splits into two one-seeded parts. Capsules are also characteristic of *Menodora*, *Schrebera* and *Syringa*. The seeds are exalbuminous, as in many members of the Verbenaceae and species of *Chionanthus*, *Noronhea* and *Schrebera* of the Oleaceae.

The testa of *Nyctanthes* is thick and heavily vascularized, a feature shared by *Jasminum* and *Menodora*. In contrast, the testa of the Verbenaceae is non-vascularized (Kapil and Vani 1966). In addition the testa of *Nyctanthes* has the outer layer of large transparent cells typical of the Oleaceae (Taylor 1945) and like in *Menodora* the testa is green and the inner layers of cells contain chloroplasts (original observation).

2.4 *Stem anatomy*

Earlier accounts of the stem anatomy of *Nyctanthes* are by Fotidar (1939), Majumdar (1941), both dealing with the vascular organization, and Stant (1952) and Kundu and De (1968), whilst Murthy *et al* (1978) reported on the xylem vessels. In the latter study features of the metaxylem were highlighted, but probably confusion arose with early-formed secondary xylem vessels in some cases, and the lateral wall pitting was erroneously interpreted, rendering this study valueless. In Stant's and Kundu and De's accounts of the xylem the fibres are said to be provided with conspicuously bordered pits and xylem parenchyma is reported to be absent, which is in contrast with our observations, thus rendering the data of questionable value in comparing *Nyctanthes* with Oleaceae and Verbenaceae. We therefore give a new description here, based on the study of four authenticated samples of *N. arbor-tristis*. The description of the xylem is in line with a forthcoming, comprehensive generic wood anatomical survey of the Oleaceae (see Esser and Van der Westen 1983, for a summary of the preliminary results).

Indumentum of glandular capitate and unicellular eglandular hairs (cf. 2.5a). *Cork* subepidermal. *Cortex* parenchymatous and with irregularly shaped crystals. Cortical bundles at the four stem corners inverted (*i.e.*, with the phloem on the internal side) to almost centric (figure 1), with a thin sheath of perivascular fibres confined to the internal side. *Perivascular sclerenchyma* of the central cylinder fibrous (phloem fibres). *Secondary phloem* with groups of stone cells and minute, irregularly shaped to acicular crystals in the parenchyma cells. *Secondary xylem* diffuse-porous, vessels numerous, predominantly in long radial multiples (of up to 8 vessels), diameter 30–85 μm; vessel member length *ca* 490 (300–890) μm; perforations simple in oblique end walls;



Figures 1 and 2. *Nyctanthes arbor-tristis.* **1.** Stem cross-section showing cortical bundle (above) and secondary xylem (below) with radial vessel multiples (× 125). **2.** Tangential longitudinal section of wood showing ray cells and libriform fibres (× 510).

intervessel pits alternate, nonvestured, 4–5 μm in diameter. Vessel-ray and vessel-parenchyma pits similar but half-bordered. Vasicentric tracheids very rarely present. Fibres thin- to medium thick-walled, *ca* 800 (470–1180) μm long, with minutely bordered to almost simple pits more numerous in the radial than in the tangential walls (libriform fibres, figure 2). Parenchyma very scanty apotracheal diffuse and paratracheal, in 3–4-celled strands. Rays 1–3-seriate; near the pith almost exclusively uniseriate and exclusively composed of upright cells; in thicker stems towards the periphery also with 2–3-seriates and including procumbent central cells (heterocellular, or heterogeneous type I). Primary xylem in the internodes in an almost continuous cylinder. *Pith* composed of thin-walled, lignified parenchyma cells.

Most characters of the above description are of common occurrence in the Oleaceae. Wood with vessels for a large proportion in multiples and with libriform fibres is typical for a group of genera including *Chionanthus*, *Comoranthus*, *Forestiera*, *Fraxinus*, *Haenianthus*, *Noronhia*, *Olea* and *Schrebera* (Esser and Van der Westen 1983). Affinity of *Nyctanthes* with the Verbenaceae is less likely because most species of the latter family are characterized by septate fibres.

The phloem anatomy of *Nyctanthes* is also compatible with Oleaceae on account of its stone cell groups (*cf.* Zahur 1959). Cortical bundles in the corners of quadrangular stems are also typical for the oleaceous genus *Myxopyrum*, although in the latter they are fully centric (a minor, gradual difference with *Nyctanthes*).

Thus stem anatomy supports an oleaceous rather than a verbenaceous alliance of *Nyctanthes*.

2.5 *Leaf anatomy*

2.5a *Leaf architecture*: In a recent paper Mohan and Inamdar (1983) demonstrated that *Nyctanthes* showed similarities with Oleaceae in all aspects of leaf architecture and venation.

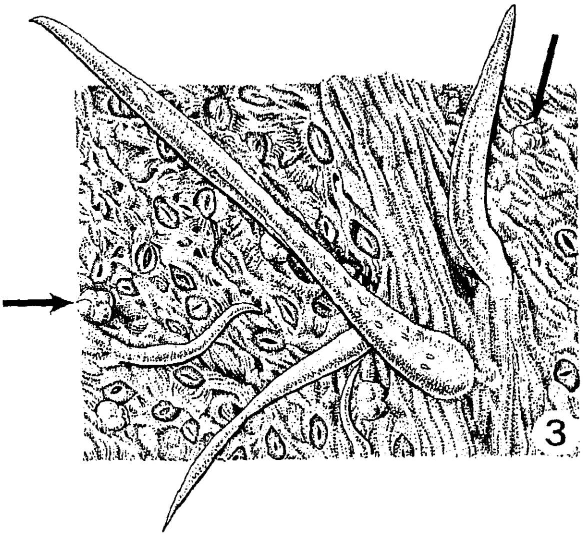
2.5b *Indumentum*: Stant (1952) described two main types of hairs for *Nyctanthes*: glandular hairs (found on both surfaces) which have a slightly depressed unicellular stalk and a head of four cells (figure 3), and unicellular trichomes (which are more frequent abaxially but are longer adaxially) having a base of eight adjacent cells which protrudes from the surface (figures 3 and 4). The former hair type is common among species of the Oleaceae, the latter is unusual especially for its length and raised hair base. The cells forming part of the hair base complex at the adaxial side are strongly sclerified and give the impression of being silicified in addition (original observation); the number of cells around the unicellular hair varies somewhat (6–9) and other cells around and below this ring (local hypodermis) are also sclerified and probably silicified. These unicellular trichomes give the upper surface its scabrid texture. The scabrid hairs of *Menodora scabra* are much shorter and hooked and have only a slightly raised base (original observation).

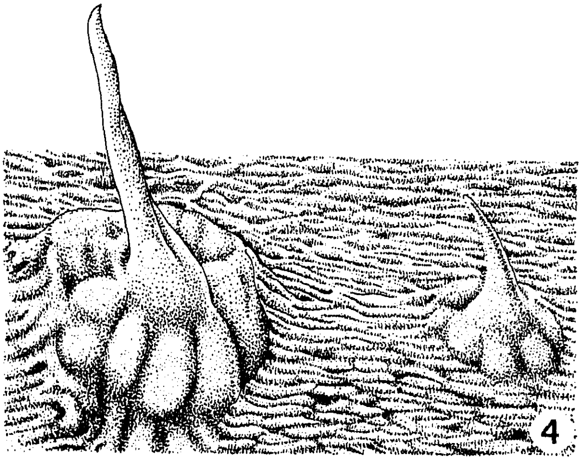
Inamdar (1967) in a detailed study of the hairs of a few species of *Jasminum* and of *N. arbor-tristis* described eight hair types for *Nyctanthes*. Of these only the fusiform eglandular type was not found in the four *Jasminum* species he examined. Unicellular trichomes are therefore not unique to *Nyctanthes* as is also apparent from Metcalfe and Chalk's (1950) account of the Oleaceae.

2.5c *Stomata*: The stomata of *N. arbor-tristis* are anomocytic, and thus typical of the family Oleaceae.

2.5d *Foliar sclereids*: Rao (1947) and Kundu and De (1968) reported the presence of sclerosed palisade cells in *N. arbor-tristis*, which occur as isolated cells or in groups of a few cells. These are short rod-like cells with thick walls and a narrow lumen. Foliar sclereids have been found in most representatives of the Oleaceae (Rao and Das 1979) but not in all (Solereder 1899); those of *Nyctanthes* are, however, atypical in not showing intrusive growth. Stant (1952) did not observe any mesophyll sclereids. In our material presence or absence varies with the specimen, probably related to absolute age of the leaf. Examination of a series of six leaves from a developing shoot showed that sclerosed palisade cells are only present in leaves which are fully expanded.

2.5e *Crystals*: Crystals are not present in the leaf of either species of *Nyctanthes* (Stant 1952; Kundu and De 1968; our own study). Although common in many species, they are





Figures 3 and 4. *Nyctanthes arbor-tristis*. Drawings from SEM micrographs. **3.** Abaxial leaf surface with vein and long unicellular hairs. Note also glandular hairs (arrows) and stomata (× 230). **4.** Adaxial surface showing hairs which cause the scabrid texture of the leaf (× 190).

not invariably present in species of Oleaceae. For example, examination of 15 species of *Chionanthus* showed that crystals were present in only seven of these species (Kiew and Che Su 1982). It may be noted in passing that the type of crystals found in some of the stem material (see §2.4) is compatible with Oleaceae, which often have minute acicular or irregularly shaped crystals in both stem and leaf.

2.5f *Petiole*: The crescent-shaped vascular system of the petiole in *Nyctanthes* which is flanked by two or three pairs of lateral traces (Fotidar 1939; Majumdar 1941; Stant 1952; Kundu and De 1968; and our own study) is also common in members of the Oleaceae (Metcalfe and Chalk 1950; Kiew 1983).

2.6 *Flower vasculature*

Joshi and Fotidar (1940) investigated the course of the vascular system from the pedicel into the ovule. For *Syringa*, *Olea* and *Ligustrum* they found that the ovule received a single bundle which ran unbranched and ended in the chalaza. In *Jasminum* and *Nyctanthes* the crescent-shaped ovular trace divided into two bundles in the funicle and then further divided until the vascular system of the ovule consisted of about 25 to 30 bundles in the integument. Kshetrapal and Tiagi (1970) noted that this strong integumentary vascular system is not encountered in the Verbenaceae.

2.7 *Embryology*

Kapil and Vani (1966) reported that *Nyctanthes* differs from the Verbenaceae in two important characters. Firstly, the antipodal cells of *Nyctanthes* are small and ephemeral, whereas in the Verbenaceae they are large and persistent. Secondly, following the system of Souèges, the embryogeny of *Nyctanthes* is of the Megarchetype III of Second Period, whereas that of Verbenaceae is Megarchetype IV of First Period. *Jasminum* is the same as *Nyctanthes* for these two characters. Devi (1975) also found that the embryology of *Nyctanthes arbor-tristis* and species of *Jasminum* is basically similar.

2.8 *Pollen*

Saxena (1975) described the pollen of *Nyctanthes* as having a thick, subtectate sexine with winding muri and wide lumen (with a maximum diameter of over 8 μm) and provided with luminal bacules. He stated that he had not found this type of pollen in his investigation of 286 species in 77 genera of the Verbenaceae. Kundu and De (1968) noted that *Nyctanthes* pollen resembled the pollen of *Jasminum* to some extent, the latter also having a reticulate surface pattern.

2.9 *Chromosome number*

The somatic chromosome number of *Nyctanthes* has been recorded as 44 (Bolkhorskikh *et al* 1969) and as 46 (Kundu and De 1968). Both these numbers occur in the Oleaceae: $2n = 44$ is recorded for *Menodora scabra* (Taylor 1945, who also recorded $2n = 22$ for two other species of *Menodora*) and *Ligustrum ibota*, *L. japonicum* and *L. tschonoskii*, *Osmanthes fortunei* and *O. sandwicensis*, as well as *Syringa amurensis*, *S. emodii*, *S. josikaea*, *S. persica* and *S. vulgaris* (Bolkhorskikh *et al* 1969); while $2n = 46$ is reported from eight genera of the Oleaceae, viz. *Chionanthus* (including *Linociera*), *Fraxinus*, *Ligustrum*, *Nestegis*, *Olea*, *Osmanthus*, *Picconia* and *Schrebera* (Bolkhorskikh *et al* 1969).

In contrast, of more than 200 species of the Verbenaceae surveyed only three species have $2n = 44$ (*Lantana camara*, *L. horrida* and *L. indica*) and one species, *Clerodendron thomsonae*, has $2n = 46$.

2.10 *Phytochemistry*

Das and Rao (1966) compared the phenolic acids of *Nyctanthes arbor-tristis* with a few species of the Oleaceae and the Verbenaceae. Of the 13 phenolic acids investigated,

eight were common to both families. *Nyctanthes* had caffeic acid present, which occurs in the Verbenaceae but only as a trace in the Oleaceae. In common with other members of the Oleaceae investigated, O-pyrocatechuic acid, phloretic acid and syringic acid were absent (they are present in the Verbenaceae) while ferulic acid was present in large amounts in both *Nyctanthes* and other members of the Oleaceae, but was present as only a trace in the Verbenaceae studied.

Hegnauer (1969) on the basis of mannitols, agreed that *Nyctanthes* is closer to the Oleaceae than the Verbenaceae. Harborne and Green (1980) found that *Nyctanthes* has the two common flavones of the family Oleaceae.

3. Discussion

The grounds for transferring *Nyctanthes* to the Verbenaceae are primarily its habit (its quadrangular stems and scabrid, dentate leaves) which is more commonly encountered in members of the Verbenaceae. However, species with these characters are also found in the Oleaceae. Apart from habit, there are no other characters which support its position in the Verbenaceae while evidence based on floral morphology, characters of the testa and integument, vegetative anatomy, embryology and palynology, chromosome number and phytochemistry all confirm its affinity with the Oleaceae.

Among the genera of the Oleaceae, *Nyctanthes* shows most similarity to *Jasminum, Menodora* and *Schrebera* (table 1) and, with *Myxopyrum* it shares a similar habit (quadrangular stem with cortical bundles in the corners and dentate leaves) as well as having ascending ovules.

The grouping of similar genera in the Oleaceae has been obscured by the division of the family into two subfamilies. *Sensu* Knoblauch (1892), subfamily Jasminoideae was small and consisted of three genera, *Jasminum, Menodora* and *Nyctanthes*, and subfamily Oleoideae was larger and more diverse and included the remaining 18 genera. Johnson (1957) reversed this situation by confining the subfamily Oleoideae to Tribe *Fraxineae* and Tribe *Oleeae*. As he commented subfamily Jasminoideae then became "merely a convenient and capacious pigeonhole for everything that is excluded from the

Table 1. *Nyctanthes* compared with *Jasminum, Menodora* and *Schrebera.*

Characters of *Nyctanthes*	*Menodora*	*Jasminum*	*Schrebera*
More than 4 corolla lobes	+	+	+
Flowers sweet scented and open in evening, night	+	+	−
Seed ascending	+	+	−
Endosperm scanty or absent	+	+	+
Integument and testa strongly vascularized	+	+	−
Testa chloropyllous	+	−	−
Leaves scabrid	+	−	−
Fruit a capsule	+	−	+
Heterostylous	?	+	+
Vessels mostly in multiples	−	−	+
Libriform fibres	−	−	+
Total number of shared characters	8	6	6

+ = presence; − = absence; ? = not known

Oleoideae." More detailed consideration of the genera he had removed to subfamily Jasminoideae, namely *Schrebera* (Harborne and Green 1980) and *Myxopyrum* (Kiew 1983) show them to be more closely related to Tribe *Oleeae.* In fact neither subfamily can accommodate the range of diversity shown by the genera of the Oleaceae and it is more meaningful to abandon the use of the subfamilies altogether and to revert to the practice of Bentham and Hooker (1876) of using tribes of equal standing which can be arranged to show the relationship between them.

Bentham and Hooker (1876) recognized four tribes: Jasmineae (equivalent to Knoblauch's subfamily as it contained the same genera, *Jasminum, Menodora* and *Nyctanthes*); Syringeae, Fraxineae and Oleineae. Johnson (1957) described four additional tribes: Fontanesieae H. Taylor & L. Johnson (split from Fraxineae); Forsythieae H. Taylor *ex* L. Johnson (split from Syringeae); Schrebereae L. Johnson (split from Syringeae) and Myxopyreae (split from Oleeae). These four tribes were accommodated within his capacious subfamily Jasminoideae. *Syringa* was placed next to *Ligustrum* in Tribe Oleeae.

Since Airy Shaw (1952) transferred *Nyctanthes* to subfamily Nyctanthoideae in the Verbenaceae, many authors (Das and Rao 1966; Kapil and Vani 1966; Kshetrapal and Tiagi 1970; Mohan and Inamdar 1983; Saxena 1975; Thorne 1983) have considered that it should be retained in the Oleaceae and Devi (1975) has suggested subfamily rank for *Nyctanthes* in the Oleaceae, as does Thorne (1983). Kundu and De (1968) accorded *Nyctanthes* family status.

Accepting that *Nyctanthes* belongs to the Oleaceae, it is nevertheless unique for this family possessing a corolla which is truly contorted and a two-seeded schizocarp. What then should be its position within the Oleaceae? Should it join *Jasminum* and *Menodora* in Tribe Jasmineae or should it be given tribal or even subfamily rank?

It is always possible to argue that a particular genus possesses unique characters that would merit tribal rank, but this would defeat the purpose of suprageneric ranking which aims to reflect relationships between genera. If a monogeneric tribe or subfamily is erected for *Nyctanthes* on grounds of its habit and fruit type, then it is equally justified to erect a monogeneric tribe for *Jasminum* (which is unique in the Oleaceae for its climbing habit combined with the possession of pinnate leaves and bilobed berries), for *Menodora*, and so on for other genera.

However, this is unnecessary as table 1 indicates that *Nyctanthes* shares an array of characters with *Jasminum* and *Menodora*, and can therefore easily be accommodated within tribe Jasmineae *sensu* Bentham and Hooker as is also shown in §4. The differences in wood anatomy between *Nyctanthes* and the other two genera of the Jasmineae indicate a higher level of specialization of *Nyctanthes*. In other tribes similar differences in the level of wood specialization also occur (Esser and Van der Westen 1983, and in preparation).

Table 1 also indicates that *Schrebera* (in Tribe Schrebereae) is more similar to Tribe Jasmineae than are the other tribes. It will, however, be interesting to see if further chromosome counts of *Nyctanthes* confirm $2n = 44$ (as $n = 11$ is the base number for *Menodora*) or $2n = 46$ ($n = 23$ is the base number for *Schrebera*).

4. Taxonomy

Tribe Jasminae Bentham & Hooker f.
Bentham & Hooker f. (1876) Gen Plant. **2** 672.

Synonyms: Nyctanthaceae Kundu and Anima De (1968) *Bull. Bot. Survey India.* **10** 407

Subfamily Jasminoideae Knobl. (1892) in *E and P. Pflanzenfam.* **4, 2** 13

Subfamily Nyctanthoideae nom. nud. Devi (1975) *Acta Bot. Indica* **3** 52; Thorne (1983) *Nord. J. Bot.* **3** 107

Subfamily Nyctanthoideae (Verbenaceae) Airy Shaw (1952) *Kew Bull.* 272

Small trees, shrubs or climbers. Leaves simple, 1–,3-pinnatisect or imparipinnate, sometimes scabrid. Corolla rather large with well-developed tube, 4–12 lobes, usually white or yellow, sweet-scented and opening in evening or night. Fruit bilobed, either fleshy or dry, or a schizocarp. Seed ascending, with thick, strongly vascularized testa. Endosperm scanty or absent.

Chromosome number: Jasminum $n = 13, 14$; Menodora $n = 11$; Nyctanthes $n = 22, 23$.

Key to the genera

1. Stem quadrangular, corolla lobes contorted, fruit a 2-seeded schizocarp, not bilobed .... *Nyctanthes*
1. Stems terete, corolla lobes imbricate, fruit bilobed, not a schizocarp
   2. Fruit a bilobed berry. Shrubs or climbers with pinnate, tri- or unifoliate leaves .... *Jasminum*
   2. Fruit a bilobed capsule. Shrubs with simple or 1–,3-pinnatisect leaves .... *Menodora*

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Increasing plant productivity through improved photosynthesis

K K G MENON and H C SRIVASTAVA
Hindustan Lever Research Centre, Andheri East, Bombay 400 099, India

Abstract. The importance of increasing plant productivity through photosynthetic route and relevance of higher chain aliphatic alcohols in promoting photosynthesis in plants, resulting in increased yields in various crops is discussed. A mixture of aliphatic alcohols (C-24 to C-34) designated as "Mixtalol" was prepared and tested as seed soak and foliar spray at 1–2 ppm. It was found that the treatment resulted in a significant increase in root length and number of laterals, shoot fresh weight and shoot and root dry weight of various crop plants. Mixtalol treatment as seed soaking of paddy increased the chlorophyll content of leaves, which was higher at younger stages of development. The seed soak and foliar spray of Mixtalol also increased Fe^{++} content of tomato and paddy shoots. It also significantly increased the rate of photosynthesis in tomato and paddy. In tomato and barley leaves, a simultaneous decrease in photorespiration rates was also observed.

Foliar application of individual alcohols, (components of Mixtalol) indicated that excepting for C-28, C-22 to C-30 increased the rate of photosynthesis. A mixture of C-24 to C-30, in the same proportion as that of Mixtalol, increased the rate of photosynthesis in paddy but Mixtalol registered a higher rate than the mixture, probably, because of the presence of still unidentified components in the mixture. Extensive field trials with Mixtalol, have shown yield increases of 14–27 % in paddy, 13–27 % in wheat, 33 % in maize, 20 % in pearl millet, 21–29 % in potatoes, 15–20 % in groundnuts and 48 % in sorghum fodder. The foliar application of Mixtalol on vegetables (tomato, brinjal, okra, beans, cauliflower, chilli, etc.) gave substantial increases in yield.

Keywords. Photosynthesis; photorespiration; chlorophyll; aliphatic alcohols; Mixtalol; *Lycopersicon*; *Oryza*; *Triticum*; *Zea*; *Pennisetum*; *Sorghum*; *Arachis*; vegetables.

1. Introduction

Plant productivity is dependent upon the interaction between genetic potentialities of crop plants and the environment in which they grow. Genetic variations, weather and cultural practice normally influence the physiological processes of the plant to control growth and yield. Unfavourable environmental factors like drought, temperature, cultural practices like soil fertility, pest attack, etc can only affect the yield by affecting physiological processes of the plant. Plant breeders produce high yielding varieties by having genotypes possessing combinations of efficient physiological processes capable of producing higher yields. An enormous amount of information has been collected on the effect of mineral nutrition, water relations, growth regulators and herbicides on various physiological processes for increasing crop productivity. However, specific attention to promotion of photosynthesis in plants as a means of increasing crop productivity has not been sufficiently stressed in agriculture. The biggest challenge for scientists in agriculture research is to find ways and means to meet world food requirements by the end of the century, when the population is expected to be 6 billion, particularly when the use of fertilizers is energy-intensive (a commodity increasingly in short supply), and, further, the genetic potential seems to be reaching a ceiling with respect to non-photosynthetic means of improvement in productivity.

Plants ingest maximally only about 2·6 % of nitrogen and 0·31 % of phosphorus from

the soil compared to over 90% of dry weight obtained through photosynthesis utilising CO_2 from air, water and sunshine (Russel 1973, table 1). Photosynthesis is absolutely essential even for the incorporation of nitrogen and phosphorus, as in proteins.

While increasing crop productivity by use of nutrients alone seems to have reached a plateau, there is enormous scope of improving the yields through the photosynthetic route. The upper limit for maximum photosynthetic efficiency for terrestrial plants has been calculated to be of the order of 6·6% (Bassham 1977, table 2), but most crop plants achieve photosynthetic efficiencies only of the order of 0·15 to 0·2% (Boardman 1980), suggesting a possibility of increasing the rate many folds more and, consequently, yields (table 3).

If the rate of net photosynthesis could be increased, the yields also would improve (Wade 1973). However, a number of authors had contended earlier that there was little likelihood of materially increasing the rate of photosynthesis in plants and the best way to increase yields was by increasing the leaf area index (Watson 1952). Nevertheless,

Table 1. Per cent content in dry matter.

	Wheat	Oats	Turnips	Cloves hay
Carbon	47·62	50·37	42·91	47·40
Hydrogen	5·47	5·85	5·49	5·00
Oxygen	40·42	37·96	42·30	37·80
Nitrogen	1·2	1·3	2·3	2·6
Phosphorus	0·22	0·21	0·31	0·28
Other minerals	5·07	4·31	6·69	6·92

(After Russel 1973)

Table 2. Maximum photosynthetic efficiency

	Per cent
Active radiation from the sun	100
Photosynthetically active radiation (400–700 nm)	43
Free energy stored in reaction per mole of CO_2	
$CO_2 + H_2O \xrightarrow[\text{photons}]{\text{eight}} (CH_2O) + O_2 = 114\ \text{kcals}$	
8 Einsteins $= 8 \times 49{\cdot}74$ kcals	
Efficiency of photosynthetic reduction (aquatic) $= \dfrac{114}{8 \times 49{\cdot}74} = 0{\cdot}286$	
Total energy conversion $= 0{\cdot}286 \times 0{\cdot}43 = 0{\cdot}123$	12·3
Canopy factor 0·80	
Respiration factor 0·67	
Upper limit for land plants $= 0{\cdot}286 \times 0{\cdot}43 \times 0{\cdot}80 \times 0{\cdot}67 = 0{\cdot}066$	6·6

(after Bassham 1977)

Crop plants	Per cent
Sugarcane	1·0
Soybean	0·16
Napier grass	1·6
Cassava	0·8
Maize	0·2
Wheat, rice, etc	0·15
Forest trees	0·06

(After Boardmann 1980).

during recent years, there has been extensive research for search of plants with high rate of photosynthesis per unit leaf area and also investigations for more efficient mechanisms of photosynthesis (Staples and Kuhr 1980). Since C-4 carbon pathway is more efficient than the C-3 pathway, efforts could be made to modify the photosynthetic machinery through genetic engineering. However, this seems to be difficult as too many processes and too many genes are involved. Another approach involves the study of the possibility of conserving carbohydrates by finding a genetic or chemical method of reducing photorespiration (Zelitch 1975, 1979; Oliver and Zelitch 1977), which according to Servaites and Ogren (1977) can be accomplished without disturbing important internal protective mechanisms. The relationship between source and sink activity-demands on plant is a complex one and our knowledge of the regulatory processes involved is limited. Plant hormones or regulators are also involved in the regulation of physiological development by their action at the site of production and also by their transport to other sites (Wareing 1977). These activities can influence photosynthesis.

2. Importance of higher chain alcohols

The importance of higher chain alcohols in the transport and transposition of monomer of sugar and the synthesis of sugar polymers is, of late, being increasingly recognised. There is considerable evidence indicating the participation of Vitamin A (Retinol—a Tetraprenol) in sugar transfer reactions. Deprivation of Vitamin A has been shown to affect the synthesis of certain glycopeptides and glycolipids in the intestinal mucosa (DeLuca *et al* 1970, 1973), corneal epithelium (Kim and Wolf 1974) and liver (DeLuca *et al* 1975). This is also attested by the fact that one of the basic functions of vitamin A is the promotion of glycostasis in tissues. Leloir discovered the presence of a dolichol (a C_{100}-100α-saturated polyprenol) in various mammalian tissues (Parodi and Leloir 1979; Pullarkat and Reha 1982). Dolichols function in glycoprotein synthesis (Wacchler and Lennarz 1976; Lennarz 1975). Dolichols have now been found widely in plants as well. Similarly, polyprenols are present in bacteria and have been shown to act as lipid intermediates in the biosynthesis of bacterial cell wall polysaccharides (Ganguly *et al* 1980). The respective functions of retinol and dolichol in the transfer of sugar to acceptor proteins are presented in table 4 (DeLuca 1977). The presence of various growth factors, probably long chain aliphatic alcohols, in

(4 isoprenol units)	(~ 20 isoprenol units)
Functions in higher organisms only	Functions in virus, bacteria, plants and animals
Direct donor of mannose to glycoprotein without the build-up of oligosaccharide on itself	Builds up oligosaccharide chain on itself, after which the oligosaccharide is transferred to the acceptor protein
The mannosyl residue incorporated into an oligosaccharide chain (which is bound to a protein) is alkali labile and is probably O-glycosidic type of linkage	The sugar moiety (in say, ovalbumin) is attached to the asparginal residue of the protein by an alkali stable N-glycosidic bond

(After DeLuca 1977).

vertebrate saliva improving plant growth has been reported (Hollenberg and Gregory 1977; Detling *et al* 1980).

The higher chain alcohols seem to facilitate transport of sugar or passage of oligosaccharide, mostly from extracellular fluid into the cell interior, by providing a handle to the sugars, thus enabling them to cross the lipid membranes of cells as well as anchor the saccharide at specific regions of the membrane for subsequent action by membrane bound enzymes (Parodi and Leloir 1979). Vlitos and Crosby (1959) had reported the plant growth promoting activity of C-18 to C-22 alcohols and their acidic esters isolated from tobacco by the *Avena* first internode bioassay. Atkinson and Allen (1966) obtained a factor from cotton wax which was highly effective in stimulating the germination of self-inhibited wheat stem-rust uredospores. Mitchell *et al* (1967) found growth stimulating substances in the cotton fibre using bean internode bioassay. An oil product derived from Rape (*Brassica napus*) and designated as Brassins (Brassinolides) was shown by Mitchell *et al* (1970) to have plant growth-stimulating action. It consisted of five components of glyceridic structure, each having 12–26 carbon atoms (Mandava and Mitchell 1972). Yopp *et al* (1981) studied the activity of brassinolide in selected auxin bioassays.

The possible role of higher chain alcohols C-20 and above in facilitating sugar transport and synthesis attracted our particular attention to these alcohols in view of their possible role in improving physiological efficiency of the plants. Around this time we came across the work of Ries *et al* (1977) at Michigan State University, who reported that C-30 alcohol (triacontanol) extracted from alfalfa meal, when sprayed on the foliage or applied in nutrient culture, increased the growth of rice, corn and barley. Subsequently, Ries *et al* (1977, 1977a) and Ries and Wert (1977b) reported increases in leaf area and dry weight of maize and paddy treated with triacontanol. In studies with maize, rice and tomato seedlings, Jones *et al* (1979) observed that several analogues (C-16 to C-32) of triacontanol inhibited the response of plant seedlings to triacontanol, particularly C-28. Erickson *et al* (1981) noted a greater response to C-30 alcohol treatment in young tomato leaves, in which photosynthesis was less sensitive to oxygen concentration than control plants. In maize, no effect of C-30 treatment on dry weight was observed.

We considered the use of a mixture of higher chain alcohols to promote plant growth and productivity for the following reasons: Firstly, even dolichols vary in chain lengths

and different chain length alcohols have been reported to be present in various natural products. Secondly, we have taken the view that in biological systems, there is rarely present a single element deficiency, and even when such a deficiency occurs, it can rarely be treated by a single element alone, *e.g.* pellagra cannot be adequately treated with nicotinic acid alone though we know it is specific nicotinic acid deficiency. Similar is the case with beriberi (B_1 deficiency), glossitis (B_2 deficiency), achromotrichia (pantothenic acid deficiency) or pernicious anaemia (B_{12} deficiency). These conditions are all treated by administration of a mixture of B Vitamins or Vitamin B complex. It is in the nature of biological treatment therefore to resolve a deficiency state or promote improvements in nutritional/physiological state, not by a single missing nutrient but by a mixture of kindred nutrients. In this regard we considered prospects for the production of mixtures of higher chain alcohols to be used to improve physiological efficiency of plants.

3. Materials and methods

Waxy by-products of agriculture were used to obtain mixtures of a spectrum of aliphatic alcohols with chain lengths C-24 to C-34. This mixture of aliphatic alcohols was designated as "Mixtalol" and has the following composition (%): C-24 Tetracosanol: 7–10; C-26 Hexacosanol: 12–16; C-28 Octacosanol: 15–20; C-30 Triacontanol: 24–30; C-32 Dotriacontanol: 11–14; and C-34 Tetratriacontanol: 4–5.

3.1 *Preparation of Mixtalol*

Required dilutions of Mixtalol for seed soak or foliar application were prepared from 1% Mixtalol, emulsified in water using 1% each of Tween-40 and Tween-80.

3.2 *Bioassay*

Two types of bioassay were conducted to study bioeffectivity of Mixtalol as given below:,

3.2a *Root length measurement*: Root length was measured with paddy seeds (variety Jaya). The selected seeds were soaked in water for 26 hr before placing. The seeds (15–20) were placed on glass plates (9″ × 6″) covered with a moist filter paper held in place by rubber bands. Each glass plate was kept in a polythene bag containing 25 ml of distilled water. Two days later, the seedlings were selected based on the length of roots and too large or too small seedlings were discarded to overcome seed vigour variance. The seedlings were sprayed with test solutions (Mixtalol at 1–2 ppm). Test solution (4 ml) was sprayed on seedlings on each plate. Water-sprayed seedlings served as the control. Four days after the spray, their root lengths were measured and mean root length for each plate was calculated. There were 8–9 replicates for each of the treatments. The results were analysed statistically for their significance using students t test. Experiments were conducted at room temperature (25–29°C).

3.2b *Fresh and dry weight estimation*: Wheat (variety HD-2189) was used for fresh and dry weight measurements. Seeds were soaked in water for 24 hr before placing on

moist filter paper in glass petriplates 6 inches in dia. The seeds were allowed to grow for four days and made uniform by removing very large and very small seedlings. Twentyfive ml of full strength Hoaglands nutrient solution was given to each plate.

The seedlings were sprayed with 1·5 ppm Mixtalol solution. The solution (5 ml) was sprayed on each petriplate. Water-sprayed seedlings served as the control. Each treatment consisted of 5–7 replicates while each plate had 30–35 seedlings.

Four days after the spray, the seedlings were harvested. They were separated into shoot and root parts. Shoot fresh weight was recorded and later both shoot and root fractions were dried separately to a constant weight at 80°C in a hot air oven. Their dry weights were recorded. The results were analysed statistically for their significance using the students t test.

3.3 *Pot experiment*

Sieved soil was mixed with farm yard manure in the proportion 2 : 1 and filled in earthen pots (12 inches in dia). Seeds of tomato (*Lycopersicon esculentum* cv. Vaishali) and paddy (*Oryza sativa* cv. Jaya) were treated with the fungicide Bavistin (0·05 %) and sown in these pots. Doses of fertilizer (NPK) were applied at the rate of 100 : 100 : 60 kg/ha for tomatoes and 100:60:40 kg/ha for paddy in two split doses. In the case of paddy $ZnSO_4$ at the rate of 15 kg/ha was also applied. Weeding and other necessary plant protection measures were carried out as required. After the emergence of the seedlings, they were thinned to a uniform stand of 4 seedlings/pot. Both treated and control pots were regularly watered. Barley (*Hordeum vulgare*) seedlings were raised in paper cups (4 inches dia) for photorespiration studies and paddy, wheat and maize for root, shoot lengths and number of root laterals.

3.4 *Treatment*

Seed soaking and foliar spray were the two treatments given. In the first treatment, seeds were soaked in 1/1·5 ppm of Mixtalol for 24 hr and then sown in pots. Seeds soaked in distilled water for a similar period served as control. In the second treatment, first foliar spray of 1–2 ppm Mixtalol was given to the drip point when plants were four weeks old. The second foliar spray of the same concentration was similarly given four weeks after the first spray.

Plants were harvested and brought to the laboratory in moistened plastic bags. Fully expanded leaves were selected and used for the estimation of chlorophyll, net CO_2 fixation, photorespiration and Fe^{++} uptake.

3.5 *Field trials*

Random block design (RBD) was followed for replicated trials of Mixtalol on different crops. The quantity of spray used was at the rate of 500 l/ha. The first spray was made when the plants were at 2/3 leaf stage and the second spray four weeks after the first. The crop was harvested at maturity, separately threshed and grain yield/plot was recorded.

In large scale field trials, the farmers' fields were chosen and were divided in 4–6 plots of one acre each. These plots were separated by bunds. Equal doses of fertilizer (NPK) were given according to recommendations. In paddy, zinc sulphate at the rate of

15 kg/ha was applied in addition. Half the number of plots chosen at random were given Mixtalol treatment and the remaining half, served as control, which were sprayed with equal quantity of water. Weeding and other necessary plant protection measures were carried out in both control and treated plots as per requirements. At the time of harvest, produce of individual plots in treated and control was threshed separately and the weight of grains and straw recorded. The yield was reported at corrected moisture content.

3.6 *Estimation of chlorophyll*

The leaves were washed thoroughly with distilled water and blotted dry using Whatman filter paper. They were cut into small pieces (about 1·5 cm long) and 0·3 g fresh leaf tissue homogenised in 80 % acetone. The extract was centrifuged at 5000 rpm for 5 min and the supernatant decanted. The residue was washed with 80 % acetone and centrifuged again. The two supernatants were combined together and volume made to 30 ml. The absorbance was measured in a spectrophotometer at 663 and 645 nm and chlorophyll *a*, *b* and total chlorophyll content calculated by the method of Arnon (1949). The results are expressed as mg chlorophyll/g fresh weight. The values are mean of three replicates.

3.7 *Carbon dioxide fixation rates*

The CO_2 fixation rates were estimated by the method of Zelitch (1974) as modified for paddy and tomato plants.

In a 100 ml conical flask with one side arm and an inlet and an outlet for passing CO_2-free air, 5 ml Tris-HCl buffer (20 mM, pH 8·5) and 0·2 ml of $MgCl_2$ (25 mM) was taken. In the side arm 1 ml of 25 mM $NaHCO_3$ and 0·1 μCi labelled $NaHCO_3$ were taken. The leaves were washed thoroughly and blotted dry. They were cut into small pieces (about 1·5 cm long). Six such pieces were randomly selected, their fresh weight recorded and then placed in the conical flask.

The flask and the side arm were stoppered and CO_2 free air (by passing through 1 N NaOH) was flushed for 90 sec. The light source was put on (1000 W tungsten halogen lamp, 1400–1600 lux) and the leaf pieces were preilluminated for 10 min. Two ml of 6N HCl was added by a syringe to the side arm through the stopper and CO_2 released. The tissues were allowed to photosynthesize for 5 min after which the process was terminated by plunging the leaf pieces into boiling ethanol. The tissue was homogenized in ethanol and centrifuged at 5000 rpm for 5 min. The supernatant was decanted and made to 20 ml with alcohol. To 0·2 ml of this supernatant 10 ml Bray's scintillation cocktail was added and $^{14}CO_2$ counted in a liquid scintillation counter (Packard). The rates are expressed as μmol CO_2 fixed per g leaf fresh weight, per g dry weight and per mg chlorophyll per hr. The values are mean of three replicates.

3.8 *Photorespiration*

The fresh weight of six randomly selected leaf pieces was recorded and then they were allowed to photosynthesize for 10 min after liberating $^{14}CO_2$ as described earlier in the method for the estimation of CO_2 fixation rates.

After 20 min (at zero time), CO_2-free air was rapidly swept through the system and

$^{14}CO_2$ released by respiration trapped in 1 M ethanolamine for 15 min during illumination. The $^{14}CO_2$ content in the trap was determined by scintillation counting. Results were expressed as μmol $^{14}CO_2$ released per g leaf fresh weight per hour (Zelitch 1974).

3.9 *Estimation of iron*

Seedling shoot samples harvested at different days were dried in an oven at 80°C for 24 hr followed by ashing in a muffle furnace at 500°C for 2·5 hr. The ash was dissolved in 25 ml of 6 N HCl and the solution was filtered. The filtrate was made to a known volume and the iron content measured by atomic absorption at 248·3 nm using appropriate standards for comparison.

4. Results and discussion

4.1 *Effect on root length, number of laterals and shoot length of plants*

The concentration of application of Mixtalol was standardised in preliminary studies using from 0·01 ppm to 10 ppm and it was found that 1 to 2 ppm gave optimum results.

Mixtalol at 1 ppm concentration when used as a seed soak of paddy, wheat, jowar and maize produced a significant increase in root length, number of laterals and shoot length (table 5).

As shown in table 6, Mixtalol at 1 ppm spray on 4-day old paddy seedling produced a significant increase in the root length (table 6).

The shoot fresh weight increased by 8–10 %, the dry weight increased due to Mixtalol treatment (table 7).

Mixtalol application increased root length, shoot fresh weight and shoot and root dry weight. The increase in root length is dependent on water uptake since cell elongation is the primary mechanism responsible for extensive growth and water is

Table 5. Effect of seed soaking with 1 ppm Mixtalol on root length, number of laterals, and shoot length of crop plants (mean of 50 readings).

	Control	Treated
Paddy		
Root length (cm)	3·07 ± 0·17	3·94* ± 0·16
Shoot length (cm)	1·59 ± 0·09	1·62 ± 0·07
No. of laterals	2·24 ± 0·44	5·61* ± 0·99
Wheat		
Root length (cm)	6·84 ± 0·23	8·3* ± 0·52
Shoot length (cm)	6·0 ± 0·33	6·5* ± 0·37
Maize		
Root length (cm)	3·24 ± 0·16	3·66* ± 0·166
Shoot length (cm)	1·50 ± 0·07	1·34 ± 0·08
No. of laterals	2·18 ± 0·18	2·7* ± 0·19

*Significant at $P < 0.05$.

Table 6. Effect of Mixtalol on root length of paddy seedlings. (Treatment: 1·0 ppm spray 4 days after germination).

Sample	No. of observations	Mean (mm)	Mean difference ± SE (mm)
Control	91	123·24	5·60* ± 1·09
Treated	113	128·84	

*Significant at $P < 0.05$

Table 7. Effect of 1·5 ppm Mixtalol treatment (spray) on fresh and dry weights of shoots and roots of wheat seedlings (Water spray served as control).

Treatment	Shoot fr. wt. (g)	Shoot dry wt. (mg)	Root dry wt. (mg)
Experiment I			
Control	0·11 ± 0·004	12·8 ± 0·19	5·6 ± 0·55
Treated	0·12 ± 0·002	14·4 ± 0·23	6·9 ± 0·15
% Increase	10·3	12·5	25·1
Experiment II			
Control	0·11 ± 0·003	15·6 ± 0·58	6·9 ± 0·54
Treated	0·12 ± 0·002	17·3 ± 0·39	8·5 ± 0·21
% Increase	8·2	10·6	23·0

ssential to maintain cell turgor leading to cell elongation. The increase in dry weight is nostly due to an increase in the level of carbon compounds, an increase easily explained y the observed increased rate of photosynthesis and decreased photorespiration rates *vide infra*). It also indicates increased mitotic activity imparted either directly or ossibly through change in endogenous levels of auxins/cytokinins due to application f Mixtalol.

.2 *Effect on chlorophyll and iron content*

Aixtalol when applied as seed soak at 1 ppm for 24 hr in tomatoes (*Lycopersicon sculentum*) and paddy (*Oryza sativa*) produced an appreciable increase in the hlorophyll content of leaves (tables 8 and 9).

The effect of Mixtalol in increasing chlorophyll content (especially chlorophyll *a* ontent) of the leaves is higher at younger stage of development of paddy plants able 9).

Soaking tomato seeds for 24 hr in 1 ppm Mixtalol increased chlorophyll content of eaves by 23·2 % in 34-day old plants, 28·6 % in 41-day old plants. However, on day 48, reated plants had only 3·5 % increase over control (table 8). Similarly Mixtalol seed oak significantly increased the chlorophyll of paddy seedlings as well (table 9), where 7·4 % higher chlorophyll *a* was recorded. There are no reports showing that hlorophyll content of leaves can be increased by application of growth regulators.

Table 8. Effect of Mixtalol on chlorophyll content of tomato leaves. (Treatment: Seed soaking at 1 ppm for 24 hr).

Age (days)	Treatment	Total chlorophyll (mg/g fresh wt.)	% Increase over control
34	Control	1·85 (±0·107)	
	Mixtalol	2·28 (±0·099)	23·2
41	Control	1·82 (±0·094)	
	Mixtalol	2·34 (±0·146)	28·6
48	Control	2·02 (±0·055)	
	Mixtalol	2·09 (±0·062)	3·5

Table 9. Effect of Mixtalol on chlorophyll content of *O. sativa* (var. Jaya)

Treatment	Chl. a	% Control	Chl. b	% Control	Total	% Control
Water (Control)	1·90 ±0·103	—	0·69 ±0·057	—	2·59 ±0·015	—
Mixtalol	2·23 ±0·160	117·4	0·67 ±0·031	97·1	2·90 ±0·189	112·4

Paddy seeds were soaked in 1 ppm of Mixtalol for 24 hr. Chlorophyll was measured on 7-day old plants and expressed as mg/g fresh weight.

Table 10. Effect of Mixtalol on Fe^{++} content of tomato and paddy shoots.

Plant	Treatment/ Concentration	Age of plants in days	Iron content (mg. g. dry wt^{-1}) Control	Treated	% Inc.
Tomato	Seed soak 1 ppm for 24 hr	29	2·35	3·90	66
Tomato	Foliar spray 1 ppm	44	1·07	1·32	23
Paddy	Seed soak 1 ppm for 24 hr	10	1·50	2·60	73

Since iron (Fe) is required for chlorophyll synthesis, the effect of treatment with Mixtalol on the Fe content of tomato and paddy shoots was undertaken. Foliar spray was done 10 days before Fe estimations in leaves. Data presented in table 10 show increased contents of Fe in leaves after treatment with Mixtalol both in tomato and paddy in line with the observed increases in chlorophyll content.

4.3 *Effect on rate of photosynthesis*

The results obtained on the effect of Mixtalol and individual alcohols on the rate of photosynthesis of tomato and paddy leaves are given in tables 11–14.

The treatment with Mixtalol, either as seed soak or as foliar spray has shown considerable increase in the rate of photosynthesis. The application of individual alcohols which are the components of Mixtalol have indicated that C-22 to C-30 registered an increase in the rate of photosynthesis except C-28 which has shown a depression. C-20 and C-22 are in very low quantity in Mixtalol (0·01 ppm). When C-20 itself was used at 1 ppm level, it had shown a depression in the rate of photosynthesis. The mixture of C-24 to C-30 in the same proportion as that of Mixtalol have also registered a higher rate of photosynthesis but Mixtalol promoted photosynthesis better. This may be due to the presence of small amounts of unidentified photosynthesis promoters in Mixtalol—the characterization of these chemicals is in progress.

Reviewing the hormonal control of photosynthesis and assimilate distribution Treharne (1982) stated that it is reasonable to suppose that some, if not all, classes of hormone present in leaves are involved in regulating photosynthesis and closely allied processes. Chloroplasts were shown to contain gibberellins (Railton and Reid 1974) and in many experiments involving gibberellin application, effects upon photosynthesis have ranged from enhancement (Gale *et al* 1974), undetectable changes in carboxylation and phosphorylation activities (Oben and Marcelle 1975), to a nil effect or depressions (Israelstam 1979). In cases of low gibberellin levels, application of GA promoted enzyme synthesis and photosynthesis (Treharne 1978). Ericksen (1981) reported increase in the rate of photosynthesis in tomato plants in air when treated with triacontanol whereas in another set of experiment, no change in the rate of photosynthesis was observed. He also observed no change in dark respiration in both the series.

Table 11. Effect of Mixtalol on photosynthesis by tomato leaves*.

Experiment No.	Rate of photosynthesis (μmol CO_2 fixed g. fr. wt^{-1} hr^{-1}) Control	Treated	% Inc.
1.	18·61	54·47	192·69
2.	19·11	61·08	219·54
3.	20·70	51·17	147·17
Mean	19·475 ±0·630	55·57 ±2·92	185·35

*Treatment consisted of 24 hr seed soak with 1 ppm Mixtalol. Leaves of 78-day old plants were used for measurements.

Table 12. Effect of Mixtalol on photosynthesis by tomato leaves*.

Treatment	Leaf photosynthesis (μmol CO_2 fixed) g. fr. wt.$^{-1}$hr^{-1}	g. dry wt.$^{-1}$hr^{-1}	mg chlorophyll^{-1}hr^{-1}
Control (Water)	27·80 ±2·05	271·15 ±18·9	10·31 ±0·71
Mixtalol (1·5 ppm foliar spray)	48·33 ±4·12	475·75 ±80·6	18·37 ±0·71
% Inc.	73·84	75·46	78·18
P value	0·001	0·01	0·001

* 28-day old tomato plants were treated with Mixtalol at 1·5 ppm as foliar spray and measurements on photosynthesis were made after 10 days of treatment.

Table 13. Effect of Mixtalol on net CO_2 fixation in paddy leaves at different stages of growth.

Age (days)	Rate of photosynthesis (μm CO_2 fixed g. fr. wt.$^{-1}$hr^{-1}) Control	Treated	% Increase in net CO_2 fixation
95	15·8	32·18	103
104	21·2	31·37	48
116	18·6	26·41	42
125	41·2	42·3	Nil

(Treatment: 1st spray at 35 days and 2nd spray at 79-day old plants (1 ppm)).
Plants were harvested on the 130th day.

Table 14. Effect of individual aliphatic alcohols, their mixtures and Mixtalol on photosynthesis in paddy leaves.

Individual alcohols	Rate of photosynthesis (μm CO_2 fixed g. fr. wt.$^{-1}$ hr^{-1}) Control	Treated	% Increase or decrease
C-20	0·83±0·072	0·74±0·083	(−) 11
C-22	0·40±0·056	0·44±0·055	10
C-24	0·46±0·070	0·55±0·060	20
C-26	1·13±0·200	1·34±0·225	19
C-28	0·54±0·070	0·54±0·062	(−) 7
C-30	0·53±0·044	0·65±0·073	23
Mixture (C-24 to C-30)	0·83±0·193	1·06±0·269	28
Mixtalol	0·10±0·027	0·16±0·028	62

(7 days after 1 ppm spray of 30-day old plants).

The increased rate of photosynthesis shown due to the application of Mixtalol in tomato and paddy leaves could be attributed to enhanced carboxylation and phosphorylation activities besides increased chlorophyll content due to its application.

4.4 *Effect on photorespiration in C-3 plants*

Photorespiration is a group of processes by which C-3 plants release CO_2 in light at the cost of photosynthates. Zelitch (1975, 1979) has shown (table 15) that C-3 plants utilise almost 50% of the photosynthates as against only 0–6% in C-4 plants.

A search for plant growth regulators (PGR) to reduce photorespiration in order to enhance net photosynthesis will contribute to increased yields, as long as it is not harmful to the plant (Walker 1980). In fact, Zelitch (1979) reported that this process could be eliminated completely with benefits in terms of yields. Zelitch (1979a) and Lawler (1981) reviewed the prospects of regulation of photorespiration in plants by PGR. Although a number of chemicals have been reported to inhibit photorespiration a simultaneous dimunition was observed in the rate of photosynthesis in the presence of O_2 but not in the absence of O_2 suggesting that carbon from photorespiratory pathway may not be wholly able to recycle back into the Calvin cycle (Servaites and Ogren 1977).

A net promoter of photosynthesis may act either by increasing photosynthesis *per se* or by depressing photorespiration (Maugh 1981). The effect of Mixtalol on the rate of photorespiration on tomato and barley leaves is given in tables 16 and 17.

Besides increasing the rate of photosynthesis, seed soak treatment with Mixtalol was effective in decreasing the rate of photorespiration of tomato leaves. Soaking seeds for 24 hr in 1·5 ppm of Mixtalol decreased the rate of photorespiration from 6·45 to 4·7 μmol CO_2 released g.fr. wt^{-1} hr^{-1} as measured 74 days after the treatment. Spraying with 1·5 ppm of Mixtalol on 5-day old barley seedlings decreased photorespiration by 30% as measured 3 days after the spray (table 16). Decreased photo- and dark-respiration rates suggest improved physiological efficiency of treated plants in conserving photosynthates.

A number of chemicals have been reported to inhibit photorespiration (table 18)

Table 15. Photorespiration in plants.

Crops	Photorespiration as % of net photosynthesis
C-3 plants	
Alfalfa	36
Potato	50
Soybean	42–75
Sugar beet	34–55
Sunflower	27–31
Tall fescue	36–47
Tobacco	25–45
Wheat	17–69
C-4 plants	
Maize	0–6

Zelitch (1975, 1979).

Table 16. Effect of Mixtalol on photorespiration by tomato leaves (seed soaked for 24 hr in 1·5 ppm Mixtalol).

Treatment	Rate of photorespiration (PR) μmol CO_2 released g. fr. wt.$^{-1}$ hr^{-1}	% decrease in PR
54-days old plant		
Control	9·74	
Treated	3·58	63·1
71-days old plants		
Control	6·45	
Treated	4·70	27·1

Table 17. Effect of Mixtalol on photorespiration of barley leaves.

	μ mole $^{14}CO_2$ g. fr. wt.$^{-1}$ hr^{-1}		
Treatment	$^{14}CO_2$ released in light (L)	$^{14}CO_2$ released in dark (D)	L/D
Control	0·654	0·412	1·59
Mixtalol	0·461	0·130	3·55
% Change	(−) 29·5	(−) 68·5	

(Foliar spray of 1·5 ppm Mixtalol on 5-day old seedling and measured 3 days later).

Table 18. Effect of chemicals on inhibition of photorespiration.

Chemicals	Inhibitory action	Dose (mM)
α-hydroxy-2-pyridine methane sulphonic acid (α-HPMS)	Glycolate oxidase	10[1,2]
2-hydroxy-3-butynoic acid (HB)	Glycolate oxidase	0·1–0·5
Isonicotinic acid hydrazide (INH)	Conversion of glycine to serine	10–20
Aminoacetonitrile (AAN)	Conversion of glycine to serine completely	0·13
Glycine hydraxamate	Conversion of glycine to serine completely	0·24
Glycidate	Glutamate-glyoxylate aminotransferase	10–20
Mixtalol	Locus of action – not investigated	0·002

(After Keys *et al* 1982).
1. Murray and Bradbeer (1971) 2. Tee and Sin (1974).

apart from our finding of the effect of Mixtalol in the depression of photorespiration but none of them has shown to increase photosynthesis.

5. Field trials with Mixtalol on different crops

Detailed experiments conducted to establish the optimum concentration of Mixtalol for both seed soaking and foliar application have indicated that 1 to 3 ppm is effective in increasing the yields of various crops. Field trials were conducted in different locations (Andhra Pradesh, Punjab, Haryana and Uttar Pradesh). Replicated trials on various crops in different seasons indicated a significant increase in yields, which is summarised in tables 19–21.

5.1 *Paddy* (*Oryza sativa*)

Mixtalol trials were conducted both for *Kharif* season (July sowing) as well as *Rabi* season (October/November sowing). The first experiment was conducted in UP at Etah during 1978, which gave encouraging results and replicated trials were then conducted in AP at different locations from 1979 through 1982. During this period, a total of 889 trials were conducted. A summary of the results is given in table 19. The yield increases during these trials ranged from 14–27 % over the control. It is interesting to note that even at high levels of productivity (*Rabi* 1981), there was an increase of 23 % in the yields following Mixtalol treatment.

In order to see the effect of Mixtalol on increasing the number of tillers/plant and grains/panicle, experiments were conducted in *Kharif* 1981 at Bapatla, AP and the results are given in table 20. It can be seen that Mixtalol increases both the number of

Table 19. Effect of foliar application of Mixtalol (1–2 ppm) on yield of paddy (*Oryza sativa*).

Season	No. of trials	Yield kg/ha Control	Yield kg/ha Treated	% Increase
Kharif 1979	23	3268	4156	27
Kharif 1980	18	4175	5036	21
Rabi 1980	228	4076	4634	14
Kharif 1981	203	5441	6597	21
Rabi 1981	415	7360	8960	23
Kharif 1982	2	4848	6033	24

Table 20. Effect of Mixtalol on tiller and grain number of paddy.

Treatment	No. of tillers/plant	Grains/panicle
Control	9·75	215·5
Treated	12·25	238·0
% Increase	25·0	10·4

(1–2 ppm spray at 35 days and second spray at 65 days after sowing).

Table 21. Effect of foliar application of Mixtalol (1–2 ppm) on yield of various crops.

Crops	Season	No. of trials	Yield kg/ha Control	Treated	% Inc.
Triticum aestivum	*Rabi* 1979–80	6	2777	3518	27
	Rabi 1980–81	299	2733	3084	13
	Rabi 1981–82	678	3948	4660	18
Zea mays	*Kharif* 1980	6	2695	3576	33
Pennisetum typhoides	*Kharif* 1980	34	2160	2592	20
Sorghum vulgare (fodder crop)	*Kharif* 1982	1	34640	51360	48
Arachis hypogaea	*Rabi* 1980–81	6	2148	2702	25
	Kharif 1981	26	1591	1833	15

tillers/plant and also grains/panicle which are the yield components. Increase in tillers is indicative of initial vigour and as an effect of first Mixtalol application and increase in grains can be attributed to increase in rate of photosynthesis and decrease in photorespiration rates.

5.2 *Wheat (Triticum aestivum)*

Research and development trials with Mixtalol on wheat were confined to UP, Haryana and Punjab. An initial trial was conducted during *Rabi* 1979 at Etah registering a mean yield increase of 27%. Large scale field trials on the same pattern described under paddy, were conducted in 9 regions of Haryana and Punjab comprising of 229 trials during 1980–81 and 678 trials during 1981–82. The mean average increase in yield was recorded to the order of 13% and 18% respectively (table 21).

5.3 *Maize* (*Zea mays*)

Replicated trials were conducted to see the effect of Mixtalol (1–2 ppm) used as a foliar spray and results are given in table 21. It is interesting to note that a mean average increase in grain yield was 33%, which could be attributed solely to higher rate of photosynthesis as maize being a C-4 plant. Yield increases have also been recorded at various coordinated trials, following application of Mixtalol (under publication).

5.4 *Jowar* (*Sorghum vulgare*)

Jowar being used as fodder, effect of foliar spray of Mixtalol on fodder yield was studied. Replicated experiments were conducted in fields near Bombay during *Kharif* 1982 and it was found that 1 ppm spray of Mixtalol at 20 and 40 days after sowing increased 48% of the fodder yield in Sundhia variety (table 21).

5.5 *Pearl millet* (*Pennisetum typhoides*)

Mixtalol was used on three varieties of pearl millet *viz* CJ 104, BJ 104 and local and a total of 34 replicated trials were conducted in different regions. Local variety responded

ter giving an average increase in yield to the tune of 24%. The overall mean average rease in the yield of pearl millet was 20% (table 21).

Vegetables

the case of vegetables, being indeterminate crops, foliar application of Mixtalol tributed dramatic increases in yields. During 1980, a large number of trials were ducted on different vegetables in two regions, namely Etah in the North and AP in th India and the mean average yield is summarised in table 22. It can be seen from

Table 22. Effect of foliar application of Mixtalol (2–3 ppm) on vegetable yields.

Year	Crop	No. of trials	Yield kg/ha Control	Yield kg/ha Treated	% Inc.
1980	*Lycopersicon esculentum* (Tomato)	7	5092	8570	68
	Solanum tuberosum (Potato)	11	8629	10468	21
	Ipomoea batatas (Sweet Potato)	1	8222	10222	24
	Solanum melongena (Brinjal)	2	3263	4657	42
	Abelmoschus esculentus (Okra)	1	4390	5660	29
	Phaseolus vulgaris (Beans)	1	1770	2026	15
	Brassica oleracea var. *botrytis* (Cauliflower)	3	11125	13765	24
	Allium sativa (Garlic)	2	3213	3612	12
1981	*Lycopersicon esculentum* (Tomato)	2	14277	22603	58
	Ipomoea batatas (Sweet Potato)	1	7800	13000	67
	Solanum melongena (Brinjal)	2	1500	2340	56
	Abelmoschus esculentus (Okra)	1	4600	8000	74
	Phaseolus vulgaris (Beans)	5	5870	8821	50
	Luffa acutangula (Ridged Gourd)	2	10992	18539	66
	Capsicum annuum (Chilli)	1	3375	5000	48
	Pisum sativum (Green Peas)	1	4530	6750	49
1982	*Lycopersicon esculentum* (Tomato)	4	5583	8611	54
	Solanum tuberosum (Potato)	4	2788	3594	29
	Solanum melongena (Brinjal)	1	7624	10861	42

the Table that tomato had increased in yield from 55 to 80 % as compared to control. All the vegetables registered a considerable increase in yield, when Mixtalol (2–3 ppm) was applied as foliar spray twice at an interval of 30 days. It was observed that the yield increases were due to more number of fruits indicating a possibility of its role in improving fruit set. The experiments on vegetables were repeated during 1981 and 1982 with similar results (table 22).

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The science behind rotational bush fallow agriculture system (jhum)

P S RAMAKRISHNAN

Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong 793 014, India

Abstract. Rotational bush fallow agriculture variously termed as shifting agriculture, slash and burn agriculture are commonly known in India as *jhum* is a traditional agricultural system of the humid tropics and is extensively practised by the tribes of the north-eastern hill region. There is a renewed interest in this agricultural system as it has so much to offer in terms of concepts and ideas to modern agricultural organization. The science behind jhum is based on intuitive experience of the farmer based on long tradition. This paper looks at the science behind jhum with particular emphasis on the ecological and economic significance of mixed cropping, recycling of resources within the system and between jhum and animal husbandry, the non-weed concept weed potential under different cycles of jhum, and nutrient cycling. The distortions brought about by the shortening of the jhum cycle to 4–5 yr is considered. Alternate strategies for development with jhum as the focal point, with suitable modifications but without the present-day distortions, have been considered.

Keywords. Rotational bush fallow agriculture; jhum; mixed cropping; recycling of resources; animal husbandry; non-weed concept.

1. Introduction

The forest farmer in the humid tropics has managed his traditional rotational bush fallow agricultural system for centuries, with optimum yield on a long term basis, rather than trying to maximize production on a short term basis (Watters 1960; Spencer 1966; Ruthenberg 1976; Soemarwoto 1975; Gleissman *et al* 1981). Forest farming variously termed as 'shifting agriculture', 'slash and burn agriculture', 'rotational bush fallow agriculture' or popularly known as 'jhum' in India is a major activity of the tribal population of the north-east. It is also practised by tribals of Orissa, Madhya Pradesh, Andhra Pradesh, Maharashtra and in peninsular India. That this form of land use has survived up to the present as a viable practice itself suggests that the system is essentially based on sound scientific principles. The ecological basis of these practices has been the subject of intensive study by our group (Toky and Ramakrishnan 1981a, b, 1982, 1983a, b; Ramakrishnan and Toky 1981; Mishra and Ramakrishnan 1981, 1982, 1983a, b, c, d, 1984; Saxena and Ramakrishnan 1984; Ramakrishnan *et al* 1979, 1981a, b). Such a thorough understanding of the ecological processes operating in the traditional agro-ecosystems of the humid tropics could form the basis for the development of a productive system with which the tribal people can identify themselves. Such a strategy could form the basis for providing a varied all-purpose diet with ecological stability in production with efficient use of family labour and recycling of natural resources. This paper looks at the science behind jhum, at the same time highlighting the present-day distortions brought about by the shortening of the jhum cycle (Toky and Ramakrishnan 1981a; Mishra and Ramakrishnan 1981). It further looks at the possibilities of future development of cropping systems with high structural and species diversity, with jhum as the focal point.

2. Multiple cropping

One of the major objectives during cropping under jhum is to capitalize upon the transient resources of the soil and to obtain as high an economic return as possible. One of the ways in which this is achieved by the forest farmer is through multiple cropping. In fact, as many as 30 or more crops are sown together in the jhum plots. In a situation where market economy is either poorly developed or does not exist at all, multiple cropping provides not only an all-purpose diet to the farmer but also meets the need of fibre to some extent. Apart from these considerations, the multiple crop cover protects the soil from nutrient losses through hydrology and also contributes through efficient recycling of resources, aspects which would be considered at length, elsewhere.

2.1 *Cropping pattern at lower elevation jhum*

The jhum at lower elevations of Meghalaya as studied in detail by us (Toky and Ramakrishnan 1981) is typical of that practiced in many parts of the north-eastern region. Here the jhum cycle (the length of the fallow period between two successive croppings) would vary from a more frequent 4–5 yr to 10–30 yr in more remote regions. The average size of the jhum plots cropped by a family of 5 to 7 members may range from 1 to 2·5 ha.

During the winter months (December–January) the undergrowth is slashed and small trees and bamboos are felled. The boles of larger trees are not felled except for the branches. Short stumps of the trees are left in tact and underground organs of different species are not disturbed. These are left behind to have a quick regrowth of the vegetation cover after the plot is fallowed. In fact in parts of Nagaland, for *e.g.*, alder (*Alnus nepalensis*) is grown in jhum fallow plots and is allowed to coppice through stump sprouts. This species apart from being a fast growing one which provides fuel and fodder also is a non-leguminous fixer of nitrogen in the soil. Therefore it helps in quicker build-up of fertility in the soil. The slash and burn operation, being laborious is often done by the farmer on a cooperative basis with mutual help from 2–3 families joined together, with men alone taking active part in this operation. In fact, this co-operative organization of the society is not only evident in sharing of labour but the very manner in which the land is allotted by the village headman to the individual family. It may be worth noting here that private land is non-existent in the traditional rural tribal society and the land belongs to the village community as a whole.

Before the onset of the monsoon, towards the end of March or early April, the dried debris is burnt *in situ* after making a fire line around the field. After repetitive burning to destroy any unburnt material, if necessary, the seed mixtures are sown. The seed mixture used would vary depending upon the jhum cycle. In one study (Toky and Ramakrishnan 1981a), the crop mixture had 8 species under a short cycle of 5 yr and up to 13 species under a long cycle of 30 yr (table 1). All the species are sown together in the same plot. Cereals form the major component under longer cycles whereas perennials and tuber crops are emphasized under short jhum cycles. Such a shift in emphasis in crop mixtures is significant. Our results (unpublished) suggest that this is related to the nutrient status of the soil. Under short cycles where the soil is relatively infertile compared to longer cycles, the farmer shifts his crop species towards those which have a better nutrient uptake and use efficiency. Such a shift towards perennial crops also give better protective cover to the soil, checking erosive losses more effectively, once the

Table 1. Crops grown and yield in the jhum plots at lower elevations in Meghalaya (after Toky and Ramakrishnan 1981a).

	Total economic yield (kg ha^{-1} yr^{-1})		
	30 yr	10 yr	5 yr
Grain and seed			
Oryza sativa	1161	378	66
Sesamum indicum	446	541	25
Zea mays	770	397	30
Setaria italica	193	23	9
Phaseolus mungo	10	—	—
Ricinus communis	5	—	—
	(21046)	(6318)	(753)
Leaf and fruit vegetables			
Hibiscus sabdariffa	44	139	96
Hibiscus esculentus	—	50	—
Capsicum frutescence	—	1	—
Lagenaria lencantha	140	81	—
Cucurbita maxima	62	—	—
Cucumis sativa	16	—	—
Momordica charantia	—	5	—
Musa sapientum	—	105	—
	(657)	(5679)	(16182)
Tuber and rhizomes			
Manihat esculenta	338	1352	690
Colocacia antiquorum	260	294	180
Zingiber officianalis	10	—	—
	(1043)	(2712)	(1556)
Silk worm			
Cocoon (silk)	4	—	—
Pupae (without cocoon)	0·2	—	—

In parenthesis is given total plant biomass (kg ha^{-1} yr^{-1})

cover is established. Besides, the shift towards tuber crops also provide higher crop yield and better economic returns to the farmer than when put under agro-ecosystem with emphasis on cereals. This is evident when one compares a 5 yr jhum cycle under lower elevation where cereals form an important component, to potato cultivation at higher elevations in Meghalaya. The shift towards potato cropping at higher elevations is thus in accordance with the shift towards tuber crops under low nutrient status in the soil. The high elevation soils, apart from being highly leached are also acidic and are developed under pine forest cover of a temperate climate which itself retards quick fallow regrowth and the consequent fertility recovery. More about these aspects are discussed below.

Seeds of pulses, cucurbits, vegetables and cereals like *Sataria italica* are mixed with dry soil from the sites to ensure their uniform distribution and broadcast. Maize seeds are dibbled at regular intervals amongst the crops. Rice is also dibbled at regular intervals. If one considers the hill slope, even the placement of the crop along the slope

gradient is such that cereals are in the middle and lower parts of the slope while the perennials and tuber crops are emphasized on the top of the slope. Such a placement pattern on the slope, apparently, is related to the nutrient status of the soil. This again suggests that in the nutrient poorer top portion of the hill slope, for *e.g.* the emphasis is on tuber and perennial crops, whereas in the nutrient richer lower part of the slope, cereals like rice are emphasized. This aspect of the crop placement pattern on a plot which not only considers nutritional requirements such as uptake and use efficiencies but also leaf area index related to optimizing photosynthesis by the crop mixtures is currently receiving attention.

Apart from meeting the cereal and tuber crop needs of the farmer, the jhum system also meets to some extent the protein needs through legumes (figure 1). The leaves of *Ricinus communis* are used for rearing young silk worm caterpillars. Older caterpillars may be fed upon the leaves of other dicot trees left on the plot. Plots after slash and burn is used only for one year unless a garden of banana or pineapple is maintained after the first year of cropping.

2.2 *Cropping pattern at high elevation potato based modified jhum*

At higher elevations in Meghalaya, where we have done much of our studies on the modified jhum, the temperature is low, soil is highly acidic and the pine tree litter are difficult to decompose and release their nutrients faster into the soil and fallow regeneration is relatively slower. For these reasons, the farmer has modified his jhum to suit local conditions (Mishra and Ramakrishnan 1981). To start with the pine trees are sparcely scattered with much undergrowth. The undergrowth is completely slashed but only a few of the lower branches of the trees are lopped. These then are arranged in parallel rows, allowed to dry and then topped over with a thin soil layer before resorting to a more controlled burn. The cropping is done on these ridges while the furrows are compacted, running in parallel alternate rows down the slope (figure 2).

The main emphasis here is on potato along with other tuber crops such as *Ipomoea batatas* and *Colocasia antiquorum*. Some cereals like *Zea mays* and legume (*Phaseolus vulgaris*) and a few cucurbits (*Cucurbita maxima* and *Cucumis sativus*) are also sown. When a second crop is done, after the harvest of the first crop during the monsoon, the winter crop is potato alone. The emphasis on potato alone during a second cropping in the same year is perhaps related to reduced soil fertility. While under longer jhum cycles as many as 9 crop species are included, under a short 5 yr cycle only 2–3 crops of which potato is important along with *Zea mays* and *Brassica oleracea* are emphasized. Again, this reduction in the number of crop species under shorter jhum cycles is related to reduced soil fertility and increased weed problem, an aspect discussed in detail subsequently. The reduced soil fertility under shorter jhum cycles is partly compensated by the farmer with either pig dung or vegetable manure under a 10 yr cycle at the rate of 600 kg ha^{-1} yr^{-1} (oven dry wt) or with an additional supplement of NPK, 1 : 1 : 1 at the rate of 10 kg ha^{-1} yr^{-1} along with 1000 kg ha^{-1} yr^{-1} of organic manure or even more in the second year.

2.3 *Significance of multiple cropping*

As discussed earlier an important consideration in multiple cropping is related to optimal use of nutrient resources in the soil by appropriate changes in crop mixtures or

Figure 1. A close view of the mixed cropping under jhum at lower elevation of Meghalaya with tapioca, maize, colocacia, legume and cucurbit species.

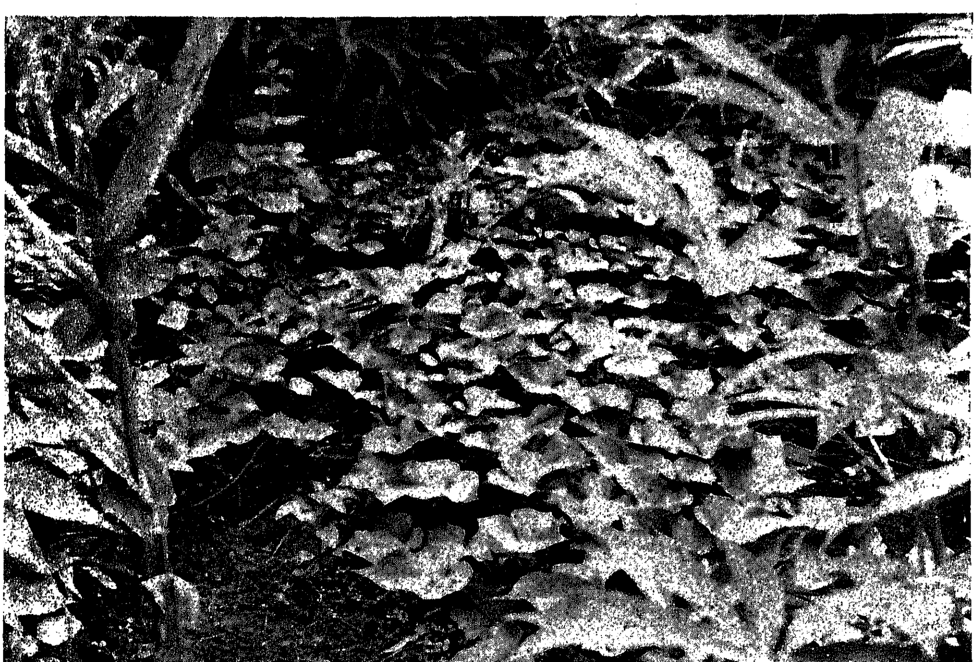

Figure 2. Modified jhum at higher elevation of Meghalaya with emphasis on potato which is being harvested. Maize crop is standing. Note the ridges and furrows running down the slope.

by manipulation of their placement, with a shift to tuber and perennial crops either with shortening of the jhum cycle or in those portions of the plot where fertility is likely to be low due to the steep gradient (20–40° angle). A high leaf area index obtained helps in checking run-off losses of sediment and nutrients after the crop cover is established. The high leaf area index also helps in optimization of photosynthesis through increased active leaf surface area and thus maximize crop yield.

A high species diversity of many tropical ecosystems (Whittaker 1965, 1972) has been considered an important factor in the stability of these natural ecosystem types (Odum 1969; Woodwell and Smith 1969; Mellinger and McNaughton 1975). Such a stability in the agricultural production system of jhum is achieved by the tribal farmer through a high species diversity in his agro-ecosystem. Apart from the fact that under a transient environment of the steep slopes of the humid tropics, such a high species diversity with a multi-layered crop canopy above would help in efficient capture of light energy and a multi-layered root mass distribution below the soil would help in optimal use of nutrient from throughout the soil profile, there are other implications of this in terms of production efficiency. Crops being sown more or less at the same time but being harvested sequentially over a long time period (table 2), provide more space for the remaining species to grow when they are at their peak growth period.

A characteristic feature of the jhum agro-ecosystem is the high rate of biomass accumulation within the system in relation to economic output (table 1). This could be up to about 18000 kg ha^{-1} yr^{-1} of total biomass under 30 yr and 5 yr cycles, the high biomass under the latter being chiefly due to the shift in emphasis towards tuber and vegetable crops. The actual economic yield component in comparison to this is only about 3500 kg ha^{-1} yr^{-1} under a 30 yr cycle and about 1600 kg ha^{-1} yr^{-1} under a 5 yr cycle with a fractional economic yield of 1/5 to 1/11 of the total biomass. Such a high organic matter production which is in agreement with the range of 16 to 22 t ha^{-1} dry weight given by Whittaker (1975) is thought to play an important part in the long term stability of the system (Sanchez 1976). With higher crop diversity, it should be possible to combine the need for increased harvestable food portion with the need for maintaining high organic biomass content in the system as a whole (Trenbath 1974). Without this organic matter production it would become necessary to constantly import costly inorganic fertilizers which are hard to come by and whose effectiveness in the face of high temperature and heavy rainfall is questionable (Gleissman 1980; Gleissman *et al* 1981; Ramakrishnan *et al* 1981a, b), particularly when the soil is extremely porous contributing to heavy infiltration losses even when the land is terraced (Toky and Ramakrishnan 1981b; Mishra and Ramakrishnan 1983a). The sequential harvest of crops would provide organic manure to the remaining crop species through efficient recycling of this important resource. The amount of nitrogen ploughed back as by-products into the system during cropping amounts to 0·6 to 0·8 kg depending upon the jhum cycle, at higher elevations of Meghalaya as shown (table 3) in one of our nitrogen budget studies (Mishra and Ramakrishnan 1984). Under a 15 yr cycle this amounts to 1/6th of the total of 4·1 kg ha^{-1} yr^{-1} non-edible biomass which goes into the system either directly or through the manure pit in the village (Mishra and Ramakrishnan 1982).

Mixed cropping as practised in this traditional agro-ecosystem is receiving considerable attention of modern agricultural scientists as a biological pest-suppressant (Litsinger and Moody 1976). The use of native varieties would probably ensure that a high degree of natural chemical defences are maintained (Janzen 1973).

Table 2. Sequential harvesting of crops on jhum plots under 30 year cycle at lower elevation of Meghalaya (after Ramakrishnan *et al* 1981a).

Species	Harvesting time
Setaria italica	Mid-July
Zea mays	Mid-July
Oryza sativa	Early September
Lagenaria spp.	"
Cucumis sativa	"
Zingiber officianalis	Early October
Sesamum indicum	"
Phaseolus mungo	"
Cucurbita spp.	Early November
Manihot esculenta	"
Colocasia antiquorum	"
Hibiscus sabdariffa	Early December
Ricinus communis	(Perennial crop)

All the seeds were sown in April.

Table 3. Nitrogen input:output budget (kg ha^{-1} yr^{-1}) for different jhum systems (after Mishra and Ramakrishnan 1984).

	Fallow cycle (yr)			
			5	
	15	10	I yr crop	II yr crop
Inputs				
Precipitation	3·6	3·6	3·6	3·6
Slash	43·6	18·6	29·3	—
Organic manure	—	8·4	14·0	25·9
Inorganic fertilizer	—	—	0·7	1·4
Weed ploughed back	0·8	2·6	3·5	3·4
By-products ploughed back	0·8	0·6	0·6	0·3
Total	48·8	53·8	51·7	34·6
Outputs				
Fire	510·2	462·1	262·8	—
Sediment	119·1	128·5	172·9	176·3
Run-off	13·0	8·2	8·9	10·0
Percolation	0·9	0·5	0·8	0·7
Weed removal	4·0	13·0	17·3	16·8
Total	690·5	643·6	482·4	213·5
Net difference	641·7	589·8	430·7	178·9

Dash represents absence of that input/output from the system.

Gleissman *et al* (1981) have considered the use of native varieties for management of modular production units based on the traditional agricultural systems of the Campesinos of the lowland tropical region of south-eastern Mexico. Under mixed cropping it is unlikely that any one of the pest populations of insects, fungi, bacteria or nematodes would reach epidemic levels due to high genetic diversity.

3. Yield pattern and socio-economics

A viewpoint commonly expressed, more often not based on data, is that jhum as an agricultural system is unviable on economic considerations compared to settled farming like terracing of the hill slopes. Even where data are available, these do not define the parameters under which these observations are made. In any study of this nature, one ought to specify the jhum cycle, the comparability of plots under consideration, the various inputs that go into the system particularly in the form of organic and inorganic manures that go into the terrace system and even the conversion factors used while expressing mixed crop yield as yield of rice or monetary returns. This confusion regarding crop yield also exists for agro-ecosystems in north-east India. In fact the rice yield under jhum was shown to be as low as 190 kg ha^{-1} (Borthakur *et al* 1978) to a high value of 1200 kg ha^{-1} (Misra 1976). Therefore the data on jhum yield at lower elevations (Toky and Ramakrishnan 1981a) and at higher elevations (Mishra and Ramakrishnan 1981) under the different jhum cycles and their comparison with sedentary valley cultivation and the terraces under comparable conditions gives an objective assessment of the yield returns. For the sake of comparisons only the monetary returns from the different systems are presented here though actual yield data are available (table 4).

One of the important conclusions of these studies is that the returns to the farmer is higher under a 10 yr jhum cycle or longer cycles compared to terrace cropping. While jhum is scientifically sound from the point of yield returns, the present-day problems are related to the distortion in the system due to shortening of jhum cycle to 4–5 yr. This apart, the quality of input into terrace is different. While the jhum system is exclusively labour-oriented which is in a way free to the farmer as it originates from

Table 4. Monetary input-output (Rs ha^{-1} yr^{-1}) into jhum, terrace and valley agro-ecosystems (after Toky and Ramakrishnan 1981a).

	Jhum (yr)				Valley
	30	10	5	Terrace	Crops I and II
Input	2616	1830	896	2542 (4544)	4843
Output	5586	3354	1690	3658	5565
Net gain/loss	2970	1524	794	1116 (−886)	722
Output/input	213	1·83	1·88	1·43 (0·80)	1·14

within the family unit itself, the input into terrace, atleast a major fraction of it, is in the form of inorganic fertilizers. If land is not a limiting factor a minimum cycle period of 10 yr is viable on economic considerations.

Apart from these, the shift in cropping pattern with shortening of the jhum cycle itself is partly based on economic considerations. Thus, if under a 5 or 10 yr cycles, the crop mixture was not altered towards perennial and tuber crops, the output from the system would have been lower. This may explain why potato cultivation under the high elevation jhum became popular when it was introduced into this region by the British in the early part of this century. Thus, a comparison of data for different jhum cycles under low and high elevations shows the returns to the farmer through emphasis on tuber (potato in this case) is 3–4 times more (table 5). However, I do realize that the comparison is not strictly valid though the broad conclusions are obvious.

Apart from the economics of it, multiple cropping provides an 'insurance policy' to the cultivators because some crops are likely to give a good return even if there is partial or complete failure of other crops. This apart, the farmer manages to get all his diverse requirements in cereals, vegetables and other tuber crops from the same site.

4. Energy efficiency

The increasing agricultural yields of the last half century were possible through industrialization of agriculture, involving large energy subsidies, sophisticated chemical control and high yielding crop varieties. Such agricultural systems are efficient in terms of human time and labour but are highly inefficient from energy viewpoint as 5–10 units of fossil fuel energy are required to produce one unit of food energy (Steinhart and Steinhart 1974). The limitations of such systems as models for development in an energy-limited world have led to renewed scientific interest in traditional systems of agriculture, such as jhum, as offering a greater ecological efficiency. Under jhum, for every unit of energy input which itself is chiefly in the form of human labour 50 or even more units of energy are harvested (Rappaport 1971; Steinhart and Steinhart 1974; Mishra and Ramakrishnan 1981; Toky and Ramakrishnan 1982).

Our studies on the three jhum cycles at lower elevations of Meghalaya (Toky and Ramakrishnan 1982) and at higher elevations of Meghalaya (Mishra and Ramakrishnan 1981) show very high efficiency values compared to sedentary terrace farming. If the cycle is long enough and land is not a limiting factor the input of solar energy to a larger area of the jhum system could offset imported fossil fuel energy which

Table 5. Monetary input-output analysis of jhum under a 10 yr cycle at lower and higher elevations of Meghalaya (after Toky and Ramakrishnan 1981a; Mishra and Ramakrishnan 1981).

	Low elevation jhum	High elevation jhum
Input	1830	3842
Output	3354	14171
Net gain	1524	10329
Output/input	1·83	3·9

would ensure harmony of the system with the environment. Even if one uses a correction factor of 1/30, 1/10 or 1/5 to calculate the effective energy output from the values given in table 6, still a 10 yr jhum cycle would be efficient in terms of energy ratio and land use (Toky and Ramakrishnan 1982). Terrace farming also involves high input of fossil fuel energy, the use efficiency of which is very low in view of heavy infiltration losses from the system (Mishra and Ramakrishnan 1983a).

Keeping the energy efficiency high, the possibilities of increased crop production has been suggested (Greenland 1975; Revelle 1976; Mutsaers *et al* 1981; Gleissman *et al* 1981) without departing too much from the traditional jhum system which has been considered as a highly evolved system for the forested areas of the tropics by many workers (Conklin 1957; Carneiro 1960; Nye and Greenland 1960; Watters 1971). Our own studies tend to confirm this, provided the jhum cycle is kept longer than 10 yr. In a wider context of the Indian agriculture, it should be possible to replace the use of imported chemical fertilizers by local resources based on bio-fertilizers using available manpower more effectively through small scale irrigation projects and so on, to have a stable production system. With a large rural population engaged in agriculture and most of them being small and marginal farmers, more emphasis on agricultural technology based on efficient recycling of natural resources, rather than a major shift to that chiefly based on chemical fertilizers, seems to be more appropriate at this juncture (Toky and Ramakrishnan 1982).

5. The non-weed concept

Weeds are wild plants that grow in highly disturbed sites (Baker 1965) and are considered to be undesirable and adversely interfering with crop yield. Therefore, in modern agriculture, considerable effort has gone into developing technology for controlling them through mechanical, chemical or biological methods. Recently, weeds have been viewed as a useful component in agro-ecosystems and therefore, in future, may play an important role in ecosystem management. Recent studies by Chacon and Gleissman (1982), Saxena and Ramakrishnan (1984) and Mishra and Ramakrishnan (1984) suggest that this 'non-weed' concept is an essential ingredient of traditional rotational bush fallow agro-ecosystems in different parts of the world.

While in many traditional systems, the incomplete removal of weeds from crop-lands may not be a deliberate husbandry practice and it may merely be due to lack of labour and poor returns from the jhum plots, all the same the weeds may be considered as

Table 6. Energy ratios in agricultural systems—jhum, terrace and valley cultivation (after Toky and Ramakrishnan 1982).

Agricultural system	Energy ($MJ\ ha^{-1}\ yr^{-1}$) Input	Output	Output/input ratio
Jhum—30 yr cycle	1665	56766	34·1
Jhum—10 yr cycle	1181	56601	47·9
Jhum—5 yr cycle	510	23858	46·7
Terrace	6509 (8003)	43602	6·7 (5·4)
Valley—I and II crops	2843	50596	17·8

useful elements in the agro-ecosystem and may often be a consequence of intuitive expertise of the farmer based on long tradition.

In north-eastern India, in the jhum plots, weeding is never total; a considerable proportion of the individuals is left in tact. Our experience with the jhum farmer suggests that he knows how intense should the weeding be so that the weed population does not interfere with the crops and yet the beneficial effects are manifest.

Obviously, one of the important roles of the weeds in the crop-lands is related to reduction in soil erosion, protection of the soil surface from solar radiation and improved soil micro-climate (Chacon and Gleissman 1982). An aspect studied in detail pertains to the role of weeds along with the crop cover in checking soil erosion. This becomes obvious when one looks at the run-off or infiltration loss patterns during the monsoon season which is the cropping period. There is a sharp peaking in the total loss of all nutrients before the plant cover is established. This is illustrated with respect to monthly losses of K, Ca and Mg in figure 3. Along with the crops, the residual weeds play an important role in checking the losses through water after the plant cover is established. The role of weeds in checking run-off and infiltration losses becomes even more dramatic in the reduction in losses of nitrogen, phosphorus and potassium through hydrology in a 5-yr old weed-dominated fallow when compared with the agro-ecosystem types of different jhum cycles (table 7). Obviously, even the exotic weeds like *Eupatorium odoratum* and *Mikania macrantha* along with others have a useful role to play in the jhum systems of the north-east.

Another important positive role of the weed lies in the recycling of the nutrients through organic manure. When the jhum farmer in the north-east does 3 to 4 partial weedings during the cropping season, at lower elevations in Meghalaya, all the weeds

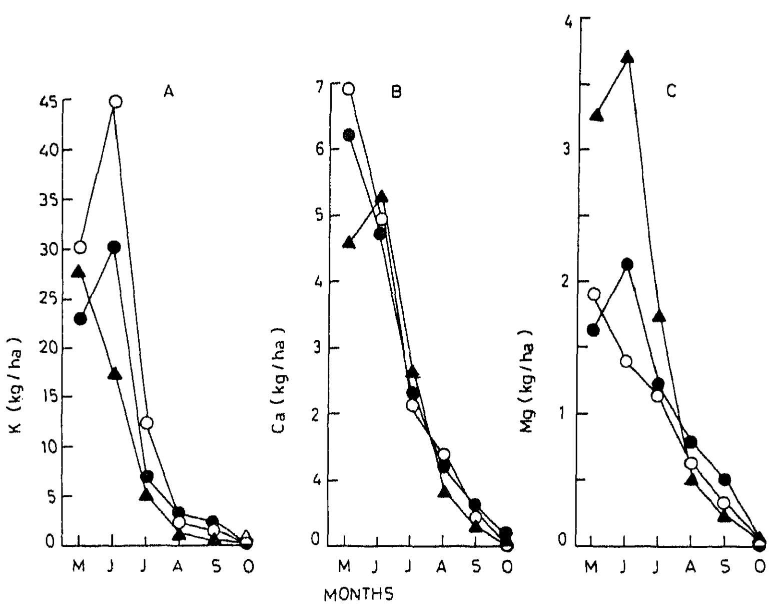

Figure 3. Monthly loss of **A.** potassium, **B.** calcium and **C.** magnesium in run-off water during the monsoon at time of cropping after the burn on sites under 30 (●), 10 (o) and 5 (▲) year jhum cycles (after Toky and Ramakrishnan 1981b).

Table 7. Nutrient losses (kg ha^{-1} yr^{-1}) through run-off and percolation water under 10 yr jhum cycle agroecosystem and a 5 yr fallow (after Toky and Ramakrishnan 1981b; Mishra and Ramakrishnan 1983a).

	10 year cycle jhum plot		5 year fallow	
	Run-off	Percolation	Run-off	Percolation
Low elevation jhum:				
Nitrate nitrogen	4·2	10·7	0·8	1·1
Available phosphate	1·3	0·1	0·1	0·02
Potassium	91·2	21·2	0·9	0·5
High elevation jhum:				
Nitrate nitrogen	1·7	0·5	1·0	0·9
Available phosphate	0·9	0·1	ND	ND
Potassium	80·1	25·8	19·6	ND

ND—Not detectable.

removed from the plot are ploughed back into the soil. Table 8 gives the weeds retained in the jhum plots (Swamy and Ramakrishnan, unpublished) along with the crops, at lower elevations in Meghalaya by the Garos. This is in contrast to what a Nepali farmer does. He is not a traditional jhum farmer. At higher elevations in Meghalaya, on the other hand, part of the harvested weed is ploughed back into the plot itself and the rest goes into the compost pit of the village ecosystem (Mishra and Ramakrishnan 1982). The compost from the pit eventually gets back into the jhum plot as organic manure. In fact, the compost pit is an essential component of the Khasi village ecosystem as it also forms an important link with animal husbandry in the form of swine husbandry by providing food for the pigs in the form of crop residues (Mishra and Ramakrishnan 1982).

In a study on the nitrogen budget of three jhum cycles of 15, 10 and 5 yr at higher elevations of Meghalaya, (Mishra and Ramakrishnan 1984) the nitrogen recycled through weeds was estimated to range from 4·8 to 20·8 kg ha^{-1} of which about 1/6th is ploughed back into the soil and the rest is routed eventually *via* the manure pit. This works out to be a good fraction of the total input into the system. It is interesting to note that the actual quantity and contribution of nitrogen through weed as a fraction of the total increased drastically with the shortening of the jhum cycle due to increased weed potential with shortening of the cycle. Not only the input of nitrogen but all other nutrients through the weed component of the jhum agro-ecosystem is significant.

Weeds are also used as a food source. In the Mokokchung District of Nagaland, for *e.g.*, the Naga tribe uses *Gnetum montanum* and *G. gnemon* as an important food resource. Bamboo shoots and wild banana are also consumed. These species are vigorous weeds in the jhum plots. Amongst the other uses, *Imperata cylindrica* is used for thatching of huts while another grass species *Thysalonaema maxima* is used for brooms. A number of plants like *Hedychium* species are also used for medicinal purposes. In fact, in the north-east the weeds associated with jhum plots used for various purposes are many depending upon the location and the tribe involved. Though this aspect of the use of the weed is not *in situ* in the jhum plots, its value in the 'non-weed' concept is significant indeed, though not in the same sense in which it is recognized as part of the agro-ecosystem itself.

Table 8. Use pattern of biomass by the Garos at lower elevations in Meghalaya (after Swamy and Ramakrishnan unpublished).

Jhum cycle (years)	Weeding	Total weed biomass produced (kg-ha)	Weed biomass ploughed back (kg-ha)	Weed biomass retained (kg-ha)
20	1	9357	7110 (76·0)	2247 (24·0)
	2	10180	8257 (81·1)	1923 (18·9)
10	1	8085	6189 (76·5)	1896 (23·5)
	2	8608	6930 (80·5)	1678 (19·5)
5	1	4378	3160 (72·2)	1218 (27·8)
	2	5780	4815 (83·3)	965 (16·7)
	3	4242	3592 (84·7)	650 (15·3)

Percentage values given in parentheses.

Apart from these beneficial effects, weeds around crop areas (Van Emden 1965; Price 1976) or within the plantings (Attleri *et al* 1977; Root 1973) can significantly alter the insect population and the resultant damage on the crop. Garcia (1980) has considered the role of weeds in the control of soil pathogens while Gleissman and Garcia (1979) have considered the possibility of controlling one weed by another.

The role of weeds in nutrient conservation, nutrient recycling and fertility recovery of the tropical traditional agricultural systems such as jhum in the north-east along with many-fold positive roles of weeds in tropical agriculture opens up a new area of agro-ecological studies. This in turn would make it possible to have a greater degree of integrated management capability (Chácon and Gleissman 1982), a capability already well-developed by the jhum farmer in the north-east India and their counterparts elsewhere. This is not to underestimate the negative role of weeds in crop production, particularly with shortening of the jhum cycle due to increased weed potential (figure 4). Though larger biomass of weeds removed from plots under shorter jhum cycles are recycled as organic manure, the weeds do pose a problem resulting in reduced yield under shorter cycles. The ingenuity of the jhum farmer lies in his ability to distinguish the 'weed' and the 'non-weed' status of the same species or set of species in his jhum plot, depending upon the intensity of the weed population in relation to the crops.

6. Why swine husbandry part of the jhum system?

Swine husbandry is an integral part of the jhum system (Mishra and Ramakrishnan 1982) and is also part of the rotational bush fallow agriculture in many parts of the world. Thus the Tsembaga farmer raises pigs to be eaten but such consumption involves religious beliefs and practices (Rappaport 1971). In fact the tribal farmer of the north-east India consumes pigs not only as part of his normal diet but makes a feast of it during celebrations related to the jhum procedures. Again the main reason why swine husbandry is part of the jhum system is because of its inexpensive maintenance costs. It is one animal husbandry practice, in the traditional sense, which is so much inter-linked with the jhum agricultural sub-system and yet makes so little demand on it.

This animal husbandry practice is based on (i) efficient recycling of resources with a

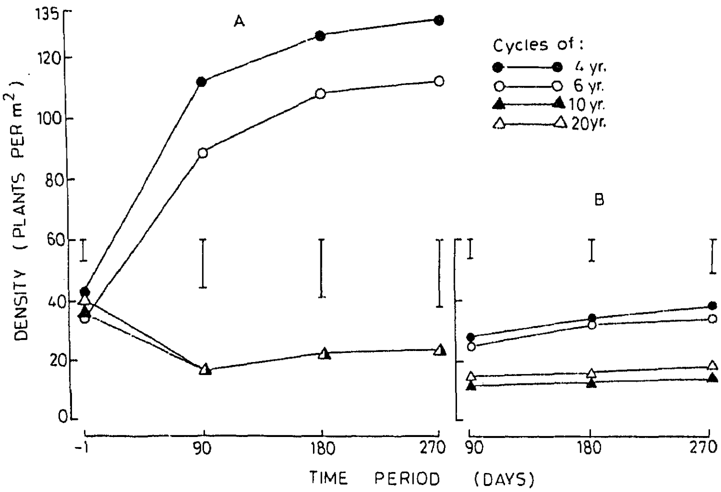

Figure 4. Density of herbaceous weeds under different jhum cycles **A.** uncropped sites; **B.** cropped sites; -1, a day before slashing the vegetation, 90, 180, 270 are the days of fallow development following the burn. Vertical lines represents LSD ($P = 0{\cdot}01$) (after Saxena and Ramakrishnan 1984). .

reasonable level of energy efficiency, two basic principles on which the operation of the jhum system itself is anchored. In a study of the animal husbandry sub-system of a Khasi village ecosystem of 20 members (Mishra and Ramakrishnan 1982), a major fraction of the total energy input is the feed, crops accounting for about 4·9 % while the crop residues plus grazing accounting for the rest of the input. Meat and dung are important outputs from the system. The output : input ratio is low if by-products and grazing are included but higher if they are excluded (figure 5).

About 70 % of the protein consumption by this Khasi village community is of plant origin, the rest being contributed by pork. While rice (*Oryza sativa*) coming out of the jhum sub-system and valley sub-system along with *Zea mays* accounted for about 70 % of the total food energy consumed, pork provided about 13 % of the total food energy consumed (table 9).

With an energy expenditure of $18{\cdot}8 \times 10^6$ MJ over a 10 yr period for raising a single pig under Tsembaga system in the New Guinea Highlands (Rappaport 1971) and with only 1·5 % of return on the food energy feed to pig meat energy according to the calculations of Pimental and Pimental (1979), this system is not very efficient though a practical way of storing excess food energy. However the efficiency of the system in the north-east India is better as the demands are lighter on the farmer. Here the animals are dependent upon crop residues, cheap feeds such as poor quality tubers which are unfit for human consumption and on browsing. Further slaughter of pigs every year rather than once in 10 yr as in Tsembaga markedly bring down the rearing costs. In short, this is one animal husbandry practice which is in harmony with the jhum system.

7. Why not terracing?

The significance of jhum as a land use in the humid tropics cannot be appreciated without a brief mention on the alternatives tried out for the tribal farmer. We have seen

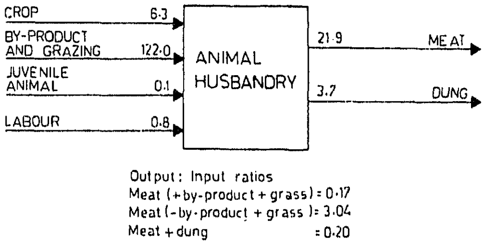

Figure 5. Energy input: output pattern and efficiency ratios for animal husbandry sub-system of a Khasi village ecosystem (after Mishra and Ramakrishnan 1982).

Table 9. Annual food and protein consumption in a Khasi village ecosystem in Meghalaya (after Mishra and Ramakrishnan 1982).

Category	Food		Protein
	Quantity (kg)	Energy equivalent (MJ)	equivalent (kg)
Solanum tuberosum	88·96	1288	4·16
Colocasia antiquorum	43·30	629	4·23
Ipomoea batatus	360·00	5965	15·59
Zea mays	342·80	5685	44·02
Oryza sativa	2567·00	42538	213·57
Phaseolus vulgaris	10·78	169	2·55
Cucurbita maxima	28·00	430	6·00
Pork	112·50	8560	93·49
Total		65264 (67383)	383·61 (321·20)

Values in parentheses are the standard requirements.

from the preceding discussions that terracing is not a viable alternative to jhum. Apart from the fact that infiltration losses are heavy (Mishra and Ramakrishnan 1983a) particularly with respect to nitrogen and phosphorus (for cations it is not so much as the slash and burn system has a heavier load of cations arising out of the ash and this is not true for terraces where burning is not done), the fertility depletions are very rapid as was observed during the second year of terrace cropping of the same site (Mishra and Ramakrishnan 1983b). In fact, the physical and chemical qualities of the soil gets so much adversely altered that the farmer very often has to leave the terrace plots after 6–8 yr of continuous cropping, as the land becomes totally desertified. In fact, our extensive observations in this region suggest that the terraces are also gradually eaten away from the periphery and that maintenance costs for these are heavy, apart from the input need for heavy dose of inorganic fertilizers which are expensive and are in short supply. Besides, the weed potential under terrace cultivation gets intensified when compared to a 10 yr jhum cycle in the same area, adversely affecting crop returns (Mishra and Ramakrishnan 1981). While valley cultivation of rice is tenable on both economic and ecologic considerations as water and nutrients are high, as the valleys form a sink for what comes through hill slopes (Toky and Ramakrishnan 1981a; Mishra and Ramakrishnan 1981), the terraces maintained closer to the valleys are viable

suggested for forest cover, the mid-portion for plantation/horticultural crops and the lower part for terraces, seems scientifically sound. However, we feel that apart from problems related to maintenance costs for terraces as viable units, the model is completely divorced from the social organization of the tribal society and may not be acceptable to the farmer in the foreseeable future.

8. Is jhum unavoidable?

A major concern of the jhum farmer has been to obtain his diverse food needs in terms of cereals, legumes, vegetables and tubers from the same plot. This becomes important for the tribal farmer because of the inaccessible terrain, cost of transportation and uncertainty of a regular and assured supply and absence of a well-developed market economy. Ideally, one would perhaps wish to avoid all forms of cereal cultivation or annual crops on the hill slopes and confine it and intensify it only in the valley lands; cultivation along the hill slopes is restricted to horticultural and plantation crops of various species for which this area is ideally suited. Such a strategy of diverting the economy would permit a permanent plant cover along the hill slopes with forestry and forestry based produces as another major supplement to the economy. If this is done, then the cereals like rice and other crops may have to be heavily supplemented by import from outside the region. Needless to emphasize here that valley system could be intensified in terms of crop varieties and number of croppings and in many other ways by improved agricultural technology.

If cereal cultivation along the hill slopes cannot be completely done away with, considering the social organization of the community and considering that jhum is a highly evolved system of land use for the humid tropics, one could consider as to how the jhum cycle can be extended to a reasonably longer time period. One could argue that the longer the cycle the better it is from the point of view of ecology and even economics. Our own extensive studies discussed in the earlier pages suggest that a 10 yr cycle is the minimum that should be attempted on both ecologic and economic considerations. We have considered economic considerations earlier. Here, perhaps a brief discussion on the ecological aspect of it in terms of environment may not be out of place.

One of the important events that occur after slash and burn is that there is a rapid depletion particularly nitrogen due to volatalization (Ramakrishnan and Toky 1981; Mishra and Ramakrishnan 1983b; Mishra and Ramakrishnan 1984) which within a few months recovers due to rapid nitrification related to higher pH and temperature on the soil surface (Ahlgren and Ahlgren 1965) and removal of allelopathic inhibitors (Smith *et al* 1968). Perhaps, under certain conditions, there could be a heavy loss of phosphorus too after the burn (Lloyd 1971; Mishra and Ramakrishnan 1983b) though there is no obvious mechanism for volatalization of this important element. However recovery occurs over a short time period during cropping. All the cations like potassium, calcium and magnesium are released in one flush after the burn. During cropping, losses occur through run-off and infiltration of water. Losses also occur due to crop removal. The total budget for one of the important elements, nitrogen, is given elsewhere (cf table 3).

After cropping, further losses of nutrients from the soil pool occur through rapid uptake and removal by the developing vegetation. The recovery process for nutrients in the soil occurs only beyond 5 to 10 yr old fallow stage (figure 6) as shown here for the high elevation jhum (Mishra and Ramakrishnan 1983b) and also observed for the low elevation system (Ramakrishnan and Toky 1981). On this consideration too continuous imposition of jhum of shorter duration than 10 yr would adversely affect the soil fertility recovery.

Another ecologic consideration for a minimum of 10 yr cycle is related to secondary successional processes and the related vegetation development. During the first 5–6 yr of fallow regrowth, the community is dominated by herbaceous weeds and subsequently by larger shrubs, bamboos and trees (Toky and Ramakrishnan 1983a, b; Mishra and Ramakrishnan 1983c, d). Our demographic analysis of weed populations (Ramakrishnan and Mishra 1981; Kushwaha *et al* 1981, 1983) suggest that these weedy species get biologically eliminated during succession after 5 to 6 yr due to change in micro-environmental conditions particularly due to reduced light available for these

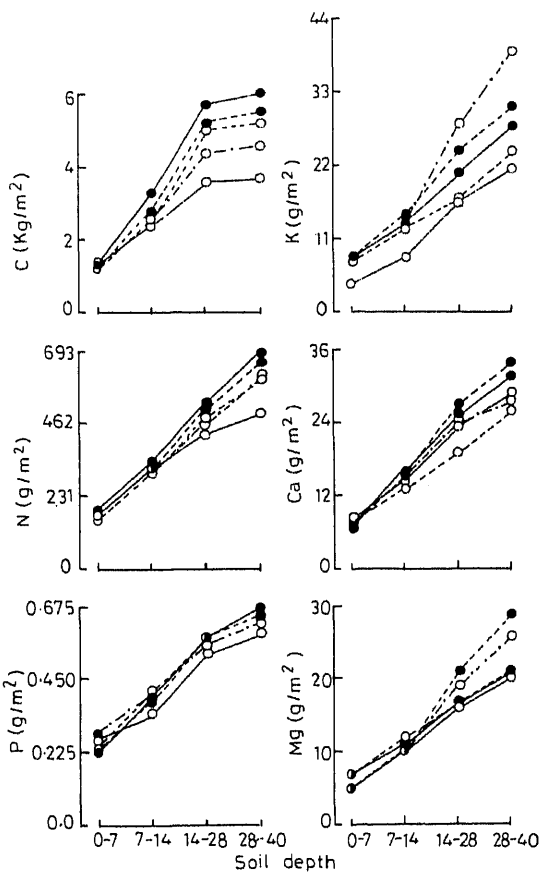

Figure 6. Changes in cumulative quantity of various nutrients (within a soil profile of 40 cm depth) during the various stages of fallow development (o–·–·–o, 0 year; o——o, 1 year; o– – –o, 5 year; •——•, 10 year; •– – –•, 15 year jhum fallow (after Mishra and Ramakrishnan 1983b)).

light demanders (Kushwaha and Ramakrishnan 1982). If the succession is not allowed to proceed beyond the 5–6 yr stage due to a shortened jhum cycle and if successive shorter cycles are imposed the succession would be arrested at the weed stage resulting in takeover of large tracts of the hill areas by weeds with consequent problems of conservation of a variety of wild germplasm of this region, which is already happening in many areas (Ramakrishnan 1984a). Therefore a jhum cycle longer than 10 yr with sufficient land area under a forest cover would ensure ecological viability.

9. Developmental strategies

With greater emphasis on valley cultivation by introducing more modern agricultural practices by way of improved varieties of crops of shorter duration of growth, one could have 2 to 3 harvests from the same site, with suitable rotation. If the pressure on the land for jhum is diverted towards a horticultural or plantation economy with village as the unit for development, then a co-operative system could be developed. One to 2 ha plot for each family for this purpose, with a number of families and contiguous village being involved on a co-operative basis—the basis which is already available in the tribal society could form viable economic plantation unit of a 100 ha or more. Fast-growing native species in small plots in the village could meet the fuelwood needs of the village community which is getting scarcer (Mishra and Ramakrishnan 1982; Ramakrishnan 1984b). Apart from a wide variety of tropical temperate fruit trees, the area is also suited for coffee, tea and rubber plantations. The possibilities are unlimited.

With such a diversion of the economy, the jhum cycle would automatically get to 10 yr or longer. Having thus increased the length of the jhum cycle, one could now think of introducing innovations into the jhum system itself at the same time retaining all the positive points associated with this traditional agricultural technology. Thus in jhum fallow plots, *e.g.* species such as *Alnus nepalensis* or legumes could be introduced which would improve soil fertility, help in quick fallow regrowth and supplement fuelwood needs, which the traditional jhum provides to the farmer to some extent. Modern agricultural technology, on the other hand, would only perhaps lead to an ever-increasing dependance on imported food products, poorer nutrition and environmental degradation, yet without achieving the production levels originally proposed, as has happened in the lowland tropical region of south-eastern Mexico (Gleissman *et al* 1981).

By having a shelter belt of forest and fruit tree species along the boundary it should be possible to check some of the losses from the jhum system through wind blow of ash during the burning season (Toky and Ramakrishnan 1981b) and also check losses through hydrology, at the same time meeting the needs of the farmer for food and fuel wood. In any land-use modification, two important social considerations that should be reconciled are: (i) that the tribal is an independent person by nature and (ii) that in spite of his being independent he works on a cooperative basis within the village sharing labour and land through the village headman, but as equal partners of the village system.

Animal husbandry in the form of swine husbandry provides much scope for development on more modern lines with better breeds. Poultry is another traditional activity of the tribal which could be better organized. These could meet the animal

protein needs of the local population and could even be exported outside the village boundary.

All these activities of a primary nature would necessitate generation of village-based low-level technology for semi-processing, atleast of the primary produce before transportation to city centres for further refinement. The low level technology could also be directed to conservation of resources within the village through better energy use for cooking or through bio-gas technology, improving upon the manure pit which is an important component in many village systems (Mishra and Ramakrishnan 1982).

The jhum farmer has always closely linked his food production system with the forests. Conservation of natural forests could be through well-planned agro-forestry and social forestry programmes designed for the village unit using native species. Further, mixed forestry systems could be developed for reclaiming damaged sites, using native tree species. Our extensive work on tree growth strategies and architecture (Ramakrishnan *et al* 1982; Boojh and Ramakrishnan 1982a, b, c, 1983; Ramakrishnan and Shukla 1982; Shukla and Ramakrishnan 1984a, b, c) is directed towards a basis for such a strategy for forest development in north-eastern India.

The major deficiency in the developmental strategy design for the tribals of the humid tropics of the north-east has been that scientists, planners and administrators have tried to impose from outside what they have considered to be good for the people of the area without trying to understand the processes that operate in the traditional ecological systems. Any developmental strategy for the future should have to be based on an understanding of the ecological processes operating in the traditional village ecosystem and with which the local people can identify. This should incorporate the empirical knowledge associated with traditional systems such as jhum.

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Forest tree improvement in India

S KEDHARNATH

Kerala Forest Research Institute, Peechi 680 653, India

Abstract. Forest tree breeding is relatively a young science. Even so, there is good evidence of its potentiality for increasing forest productivity and quality of the forest produce. The basic scheme for forest tree improvement involves selection of superior parent trees, assembling them as clones in seed orchards in special designs to promote maximum cross pollination among the different clones and reduce inbreeding. Interprovenance and interspecific hybridisation are also resorted to in special situations. Forest tree improvement work through selection and breeding has been in progress in India for the last nearly two decades. Some of the achievements and strategies used are briefly reviewed.

Keywords. Forest tree breeding; seed orchards; teak; eucalypts; pines; red sanders; semul; breeding strategies; interprovenance; inter-specific hybridisation

1. Introduction

Forest tree breeding *per se* is comparatively a young science which had its recognizable start about five decades back. Sweden was the pioneer in this venture and others followed suit slowly. Today every country, big or small, developed or developing, has active programmes on forest tree breeding. In some of the developed countries wood-based industries have invested money in such programmes with great expectations. Fortunately, there is sufficient good evidence today which indicates that such investments are sound and can pay rich dividends. In fact in recent years the research on the economics of tree improvement programmes has shifted its emphasis from programme justification to programme optimization.

Intensive management through artificial regeneration and establishment of plantations or what may be termed as 'man-made forests' are increasingly being resorted to in many countries. This naturally offers a good opportunity for not only better management of the conditions under which the trees are to grow but also of choosing appropriate genotypes which will not only have the capacity to exploit to the best advantage the environment provided but which can often be tailored to meet the specific needs. Since the area of potential production is often large even small improvements in productivity may be very significant at the national level both in terms of social benefits and production of raw material.

In this paper the general strategy that is followed in forest tree improvement and the work currently in progress in India in this fascinating field are briefly reviewed.

2. Tree improvement strategy

2.1 *Population improvement*

Exploitation of available natural genetic variability within the species is the first step in all selection and breeding work.

A characteristic feature of all living organisms is the immense natural variability they exhibit for various characters in most populations. Broadly three types of variation may be recognised (i) random variation from tree-to-tree on the same site, (ii) variations in the average of certain characters of all the trees in one locality or site when compared to the average of all trees in another locality (sometimes called local variation) and (iii) average variations in trees from widely different parts of the species range (often referred to as geographical variation or racial variation). In a regeneration programme it is essential first to identify the best adapted and productive seed origin or provenance for the species concerned. The thumb rule in forestry is "use seed from the local source until some other source or provenance has been proven superior to the local one". Superiority of new seed sources should be assessed by well laid out provenance trials.

Having identified the right provenance one can then exploit the tree-to-tree variation in economic traits for selection of superior individual trees. This is known as 'plus tree' selection. Plus trees may be defined as outstanding individuals occurring in natural stands or in even aged plantations combining in themselves a number of desirable features. As is to be expected such trees occur in low frequency and so may appear hard to find. But they do exist. These trees form the foundation for tree improvement by selection. Kedharnath (1982) has briefly reviewed the different methods employed in plus tree selection. Plus trees which are progeny tested and approved as good are called 'elite trees'. The number and type of characters used as selection criteria vary with the species but some of the most common characters used for selection are good growth vigour, superior height growth, superior diameter growth, good pruning ability, straight cylindrical bole, narrow compact crown, resistance to important diseases and insect pests.

The selected plus trees are then assembled as clones in special planting sites and they are called seed orchards. This is meant for mass producing quickly regular crops of genetically improved seeds for use in raising new plantations. These orchards require special management practices to enhance flower and seed production. Also, in the orchard the ramets of the different clones are so planted that there is very little inbreeding and maximum cross breeding between ramets of different clones in various combinations are favoured. Special planting designs are used for this. Usually grafting is resorted to using scions collected from the top one third of the flowering crown of the plus trees. This increases the probability of early flowering in the grafted plants. In some cases rooted cuttings can also be employed if graft incompatibility is a constraint. Seed orchard approach of mass producing genetically improved seeds in forest trees is so far the best publicised and most widely practised method in all the countries. This is most suitable for tree species that are normally cross pollinated. Also, to keep the genetic base sufficiently broad at least 20 clones should be used in an orchard. Theoretically there is no upper limit to the number of clones that can be used in an orchard. But the orchard should be large enough in area so that various possible cross combinations among the clones used could be realised.

2.2 *Exploitation of hybrid vigour or heterosis*

Synthesising F_1 hybrids between selected provenances of a given species could be resorted to if some specific combination manifest hybrid vigour. Mass production of such F_1 seeds could be achieved through a seed orchard programme where selected clones of two provenances may be planted. The same approach can be followed for

obtaining F_1 hybrids between two selected species if there is *prima facie* evidence of hybrid vigour.

3. Work on tree improvement in India by selection and breeding

Organised work on breeding of forest trees was started in India in 1960 at the Forest Research Institute, Dehradun in some selected tree species of economic importance. The detailed programme of work to be initiated in forest genetics and tree breeding was presented at the Tenth Silvicultural Conference held at Dehradun by Kedharnath and Raizada (1961). The Forest Genetics Branch of Forest Research Institute, Dehradun in collaboration with the State Forest Departments have been active since 1960 in carrying out this programme of work and good progress has been registered in the case of teak (*Tectona grandis* L.f.) Chirpine (*Pinus roxburghii* Sarg.), Semul (*Bombax ceiba* L.) and in some species of eucalypts. The Kerala Forest Research Institute, Peechi (Kerala), the Department of Forestry, H P Krishi Vishwa Vidyalaya and the Faculty of Forestry of Tamil Nadu Agricultural University have been making very useful contributions in this field of work. Some of the important contributions in the area are briefly reviewed below.

3.1 *Teak (Tectona grandis L.f.)*

Teak is one of the most durable and valuable timber species and belongs to the family Verbenaceae. It has 36 as its somatic chromosome number (Kedharnath and Raizada 1961). It is native to the Indo-Malayan region and occurs naturally in some parts of India while in other parts it has been successfully introduced. Presently teak is being raised in plantations on a large scale. Approximately one lakh hectares are being planted annually. The objective of tree improvement programme in teak has been to produce by selection and breeding superior stem form, superior rate of growth in height and diameter, freedom from fluting, buttressing and epicormic branches, resistance to leaf skeletoniser (*Eutectona machaeralis* syn. *Pyrausta machaeralis*) and leaf defoliator (*Hyblaea purea*). There is general evidence of inherent variation in this species for all the characteristics stated above (Kedharnath and Matthews 1962).

Work on the selection of plus trees in the species was started in the year 1960 and todate about 700 plus trees are available for use in establishing clonal seed orchards. Simple budding and/or sometimes cleft grafting technique standardised for this species by Rawat and Kedharnath (1968) has been used for clonal seed orchards and germ plasm banks that have been established in a number of states. The first experimental clonal seed orchard in this species was established at New Forest, Dehradun. Studies carried out on early growth performance of 20 clones revealed considerable variation between clones (Kedharnath *et al* 1970). These differences have persisted in later years also. Similarly observations recorded over the years on the relative resistance/susceptibility of the different clones established in the germ plasm bank and in the clonal seed orchard at New Forest, Dehradun to two leaf infecting fungi—*Olivaea tectonae* and *Caldariomyces tectonae*—under natural conditions of infection showed consistent reaction. Some were absolutely resistant, some were very susceptible while some were moderately resistant. These two diseases are not economically important. In respect of studies on variation in fibre length carried out using trees from a replicated provenance

experiment revealed significant geographical and tree-to-tree variation in this trait (Kedharnath *et al* 1963). Testing of some of the clones of *T. grandis* and a related species *T. hamiltoniana* under controlled conditions for variation in resistance to *Eutectona machaeralis* showed that there is significant variation among the clones tested (Kedharnath and Pratap Singh 1975).

Studies carried out on vegetative propagation such as rooting of cuttings, grafting, budding etc have shown that it is a favourable material for cloning. There has not been any indication of graft incompatibility in *T. grandis*. Furthermore, budding on naked stumps (with about 15 cm length of root and about 3 cm of stem) just above the collar region gives very good take. Customarily such budded stumps are planted in polypots and the new sprout emerges in 15–20 days. The best time for such budding appears to be March–April. In June–July they are transplanted in the seed orchard site. More recently attempts have been made for clonal multiplication through *in vitro* tissue culture techniques and very good success has been obtained by Gupta *et al* (1980) at the National Chemical Laboratory, Pune. The important aspect of the above work is that mature excised terminal buds from field grown trees about 100 years old have been induced to form multiple shoots on a defined medium. Individual shoots were later made to develop roots on a low salt medium, containing three auxins. The plants so obtained have been later transplanted in pots and finally in the field. This technique offers a good method for cloning. But this is yet to become popular with foresters for large scale adoption in clonal seed orchard establishment.

All the teak-growing states are establishing clonal seed orchards for teak. It has been estimated that if one plants 156 grafts per hectare at 8 × 8 m espacement 1280 ha of seed orchards will be needed. Roughly 2 grafted plants will suffice to give enough seeds (3 kg each) to plant a hectare. About 800 ha of seed orchards have been established so far.

Progeny testing of the plus trees is an essential step to know the breeding value of the plus trees. Open-pollinated seeds from the plus trees have been collected and used in some states like Tamil Nadu, while some others have taken advantage of the early flowering of the different clones in the orchard and collected seeds under open pollination clone-wise and raised seedlings for establishing progeny trial. One interesting observation reported by Kedharnath (1973) regarding some of the seed orchards pertains to early flowering observed in many clones, non-synchronous flowering among some clones and production in general of good well-filled seeds with a very high percentage of germination. Intensive management of the orchards should be helpful in enhancing flower and seed production.

Some of the states like Andhra Pradesh, Gujarat, Kerala, Madhya Pradesh, Maharashtra and Tamil Nadu have made good progress in the selection of plus trees and in establishing clonal seed orchards. Now efforts are in progress to manage the orchards intensively so as to enhance flowering and fruit production.

Some of the teak plus trees of Tamil Nadu have been used as experimental material for gel electrophoresis studies to identify easterase bands (Kumaravelu 1979).

Two other species, *Tectona hamiltoniana* Wall. and *T. philippinensis* Benth. and Horn. f. ex. Merr. are known under the genus *Tectona*. They are not very valuable as timber species. *T. hamiltoniana* has 36 as its somatic chromosome number. This species has been introduced in Dehradun from Burma. Trees of this species appear to be comparatively free from attacks of leaf skeletoniser and defoliator. Exploratory crosses were therefore attempted between *T. grandis* and *T. hamiltoniana*. This cross, however, yielded only shrivelled seeds and failed to germinate. Embryological studies revealed

that fertilisation does take place in this cross but the hybrid embryo aborted very early. It should be possible to realise this hybrid by employing embryo-culture technique. Grafting work carried out with these two species *viz T. grandis* as stock and *T. hamiltoniana* as scion showed that the grafts are able to survive for about six years under Dehradun conditions and then graft incompatibility manifests itself. This is not surprising, considering the fact that *T. grandis* has ring porus wood while *T. hamiltoniana* has diffuse porus wood. The same graft combination has been carried out also at Kerala Forest Research Institute, Peechi which are 4 years old now and have grown well. It remains to be seen as to how soon late graft incompatibility will manifest itself in these. It is interesting in this connection to point out that studies carried by Gottwald and Parameswaran (1980) show that the general properties and anatomical features of the wood and bark, together with the leaf trichomes, are markedly different between *T. grandis* (of sect. *Tectona*) on the one hand and *T. hamiltoniana* and *T. philippinensis* (of sect. Leiocarpae) on the other. Perhaps a taxonomical revision of the genus may suggest retention of a single species under the genus *Tectona, viz T. grandis* and the other two taxa *T. philippinensis* and *T. hamiltoniana* be shifted to another new genus or put under some other already existing genus like for instance *Gmelina*.

3.2 *Eucalypts*

Three species of eucalypts in particular, *Eucalyptus tereticornis*, *E. grandis* and *E. globulus* are in use for raising large scale plantations. *E. globulus* is mostly used in the Nilgiris in South India and no work on the genetic improvement of this species has been initiated so far. In respect of the other two species a lot of research work has been done for the genetic upgrading of the species. The provenance of *E. tereticornis* usually referred to as, 'Mysore Gum' or sometimes as 'Mysore hybrid' is the one that is in use in most of the states for raising large scale industrial plantations. It is also in use in agroforestry. *E. grandis* is usually raised in higher ranges of western ghats particularly in Kerala and to a small extent in Tamil Nadu. These two species are worked on a short rotation of 8 yr. While in many areas it has given good yields, in some areas, however, the yields have been rather poor. Work on provenance testing is in progress in a number of states.

Differences in rate of growth and susceptibility to *Cylindrocladium* blight has been reported by Jayashree *et al* (1984) from a study of 39 provenances representing 15 species. A toxin bioassay method for assessing relative susceptibility of eucalypts to pink disease caused by *Corticium salmonicolor* has been reported by Sharma *et al* (1984). They screened 23 eucalypt entries and observed significant variation in their susceptibility.

Plus tree selection and establishment of progeny trial has been taken up in *E. grandis* and *E. tereticornis*. Vegetative propagation by rooting of stem cuttings has not been very encouraging for large scale use. Clonal propagation by grafting has also not been very encouraging because of late manifestation of graft incompatibility. Thus, there has not been much enthusiasm for establishing clonal seed orchards. However, it is hoped that with the recent reports on the success achieved in obtaining plantlets from meristem culture in *E. citriodora* by Gupta *et al* (1981) and in *E. grandis* by Lakshmi Sita *et al* (1984) there will be enthusiasm to use this approach for establishing clonal seed orchards.

Valuable information on various genetic parameters has been reported from

E. tereticornis and *E. grandis* by Kedharnath and Vakshasya (1977), Kedharnath (1982a) and Krishnaswamy *et al* (1984).

A number of spontaneously occurring interspecific hybrids have been identified and studied in India (Kedharnath 1980). These include *E. camaldulensis* × *E. tereticornis*, *E. citriodora* × *E. torreliana*, *E. grandis* × *E. tereticornis*. These hybrids manifest good hybrid vigour for growth and volume production. It would be very beneficial to multiply them clonally and establish plantations using the tissue culture approach. Also, experimentally synthesised hybrids have been evaluated by Venkatesh and Sharma (1977). F_1 hybrids from some of the cross combinations exhibit good hybrid vigour.

3.3 *Pines*

Till very recently only four species of pines were known in India—*Pinus roxburghii* Sarg., *P. wallichiana* Jack., *P. kesiya* Royle ex Gordon and *P. gerardiana* Wall. Now one more species *P. bhutanica* Grierson, Long and Page, has been recorded from Arunachal Pradesh. This species was collected from Arunachal Pradesh by Naithani and Sahni in 1977 (Naithani and Bahadur 1981). The same species has been collected from Bhutan by Grierson *et al* (1980) and given the name *P. bhutanica*. It is a five-needle pine.

Pinus roxburghii the low level pine confined to the monsoon belt of the outer Himalaya from Bhutan to North Eastern part of West Pakistan is a valuable pine for its oleoresin and also timber. It grows in lower elevations generally up to 1830 m. Troup (1921) recognised nine provenances on the basis of growth characteristics. Considerable variation in oleoresin yield was found among the trees in the different provenances growing at New Forest, Dehradun in a provenance trial. In some provenances there were high-yielders of 4 to 7 kg. A programme of breeding for improving oleoresin yield was suggested by Kedharnath (1971). A number of plus trees specifically for high resin yielding character were selected in the State of Uttar Pradesh. Clonal propagation techniques by Cleft grafting in the succulent region with 85–90% graft take was worked out for this species (Kedharnath *et al* 1979). Additionally, the grafted plants can be used for further clonal multiplication by air-layering. A very high percentage of rooting response was obtained in airlayering trials carried out by Kedharnath and Dhaundiyal (1963). Successful rooting of stem cuttings using hormones and mist tent has also been reported from Himachal Pradesh Forest Department by Gupta (1979) and from the Forest Research Institute, Dehradun, by Bhatnagar (1979). Plus trees in this species have been selected based on characters such as good growth, stem form, straight cylindrical bole etc. by Khosla *et al* (1979) and Uniyal and Thapliyal (1979). The stage is now set for establishing clonal seed orchards. Additionally, valuable genetic information in this species has been reported by Snehalata Chawla (1977). Using an open-pollinated progeny trial she assessed the natural variation in morphological, growth and wood characters. She has also obtained heritability estimates for the various traits and correlations both phenotypic and genetic amongst various wood characters.

Studies on sensitivity of seeds of different seed origins of this species to acute gamma radiation have been reported by Upadhaya and Kedharnath (1974). When air dry seeds were used as experimental material the LD 50 for germination ranged between 3·31 and 9·12 KR. Two provenances were studied at 10 and 30% moisture content of seeds. In

one case the LD 50 came down to 7·50 KR from 9·12 KR when the moisture per cent of seeds was increased to 30 %. In the second case, the increase in moisture content to 30 % did not alter the LD 50.

The karyotypes of some of the pine species of India have been examined in detail by Mehra and Khoshoo (1956), Kumar *et al* (1966) and Upadhaya and Kedharnath (1970).

Pinus kesiya, the pine which occurs in the Khasi hills of Assam has also been taken up for genetic improvement at the Forest Research Institute, Dehradun. A provenance experiment has been laid out at New Forest, Dehradun and is being assessed regularly. The same test had also been laid out in a number of states. Additionally, a cross between *P. kesiya* and *P. merkussi* was attempted over three years. All the seeds obtained were shrivelled and they failed to germinate. In one year three viable seeds were obtained. But soon after germination the seedlings died. Thus there was no opportunity to confirm the hybridity of the seedlings.

The blue pine of Himalaya, *Pinus wallichiana*, is a soft pine which is valued very much both for its timber and oleoresin. As a prelude to initiating genetic improvement work on this species variation has been studied by Dogra (1972). He has recognised seven altitudinal provenance types. Four of these are adapted to the outer moist and inner dry north-west Himalaya; and three to the outer wet, middle moist and inner dry eastern Himalaya. The major blue pine forests grow in Kashmir, Himachal Pradesh, Uttar Pradesh and Nepal. Bhutan is the major blue pine area of the east. According to Dogra (1972) a weak reproductive barrier exists between the blue pine populations growing at lower and higher altitudes of both moist and dry zones but a strong reproductive barrier is functional between the moist and the dry arid zone blue pine of Himachal Pradesh.

The additional importance of blue pine is its resistance to blister rust caused by *Cronartium ribicola* to which the two American pines *Pinus strobus* and *P. monticola* are highly susceptible. *P. wallichiana* has been used in crossing programme with the two American species cited above and resistant hybrids manifesting hybrid vigour have been realised in USA.

3.4 *Introduced tropical pines*

Pinus patula, has been successfully introduced in West Bengal and at Kodaikanal and Ootacamund in Tamil Nadu. It is a promising species. But no work on the genetic upgrading of the species has been initiated so far in India.

Pinus caribaea has been successfully introduced in a few States. The three varieties *P. caribaea* var. *caribaea*, *P. caribaea* var. *hondurensis* and *P. caribaea* var. *bahamensis* are included in the various trials. A number of provenances of these varieties are also under trial in a number of states.

Field-grafting trials with *P. caribaea* has been carried out in India by Kapoor and Kedharnath (1976) and the time of the year best suited for field grafting has been ascertained. This should facilitate taking up work on the establishment of clonal seed orchard for this species.

3.5 *Semul (Bombex ceiba L.)*

Semul is one of the valuable indigenous soft wood species which is in great demand for use in the match industry. The annual requirement of this wood by the match industry is of the order of 2 lakh tonnes. This tree belongs to the family Bombacaceae and has a

somatic chromosome number of ca.72. Semul is widely distributed on the Indian main land while the related species *B. insigne* Wall. is confined in its distribution to the Andamans, Western Ghats and Assam. The objective of tree improvement work in this species is to evolve varieties which will be fast growing with good stem form, narrow crown and without buttresses. In nature, in some areas the trees are subject to heavy attacks by shoot borer (*Tonica niviverana* Walk). So, incorporating resistance to this pest also forms one of the breeding objectives. Also, it is known that Semul from some areas particularly that growing in Assam is valued more by the match industry because of the quality of wood. 'Plus trees' of Semul have been selected from those growing in Assam and in Uttar Pradesh and search for plus trees from other areas are in progress. A small clonal seed orchard has been established at Ranipur, 60 km from Dehra Dun using the simple grafting technique worked out for use with this species by Kedharnath and Venkatesh (1963). The grafts in the species flower the very next season after grafting if the scions had been carefully selected (Venkatesh and Arya 1967). Since each fruit contains 200–300 seeds and the percentage of germination of seed is very high, it has been estimated that half a hectare of seed orchard can yield enough seeds to plant up 500 hectares (Venkatesh 1970).

The detailed observations taken on the flowering and fruiting in the different clones in a half acre clonal seed orchard established at Ranipur, in Uttar Pradesh appear very promising from the point of view of good seed yield (Venkatesh and Arya 1967).

The chromosome number in semul has been reported from meiotic and/or somatic counts by Baker and Baker (1968), Mehra and Sareen (1973) and Sareen *et al* (1980). Somatic numbers of 72, 92, and 96 have been recorded. This would mean that both hexaploids and octoploids are present in the species if we assume that the basic chromosome number is 12. It would be interesting to raise a progeny trial as well as a clonal trial from these different chromosome number trees and assess their performance for growth and any other special attributes it may have such as resistance to drought, insect pests and also wood quality.

3.6 *Red Sanders (Pterocarpus santalinus L.f.)*

Red Sanders is a very slow growing species confined to a small region in South India. It belongs to the family *Papilionaceae* and has 24 as its somatic chromosome number. The heavy, dark claret red heartwood has been in use for centuries for carvings, doll making etc. In recent years, a variant in this species which has wavy grained wood has leapt into sudden prominence because it is highly valued in the export market. Trees with this variant character occur at very low frequency in nature and they seem to show no apparent morphological differences by which they could be easily recognised from the normal grained trees. However, such individuals can be recognised from surrounding normal trees by blazing the sap wood, because the sap wood also shows the characteristic wavy grain. Since both normal and wavy grained trees occur in the same general areas of dry sites with poor shallow soils, it is unlikely that this character is entirely controlled by environmental factors. If it is an inherited character, then the low frequency of its occurrence in nature would appear to indicate that the gene for this character is present in a low frequency in the population or the character is conditioned by multiple genes. As a first step to increase the frequency of occurrence of trees with this variant trait, such trees have been identified and assembled as grafts in a clone bank. These can then be asexually multiplied and a plantation established. Seeds have been

collected from individual trees showing the wavy grain trait in the wood to raise half-sib progenies and scoring variation among and within the progenies in growth and other characters such as internode number and average internode length. It is anticipated that there will be segregation for two kinds of seedlings in each progeny—one normal looking and the other showing stunted growth with shorter internodes. This second category of seedlings may have a high probability of yielding trees with wavy grained wood. From the work carried out in Sweden on wavy grained trait in Birch, there is evidence that this trait is genetically controlled and the frequency of recovery of such plants in the progenies of trees with this trait varies.

The variation of this trait from pith to periphery in a tree and between trees has been studied from wood core samples taken from trees at breast height. This revealed significant variation in the intensity of waviness from pith to periphery in individuals and also between trees. This information is now being used to select the most desirable trees for use in a seed orchard programme. It is anticipated that this orchard will produce seeds which would in turn yield plants that have a high probability of showing wavy grained wood. Variation in fibre morphology and the growth of grafts from different clones have been studied (Kedharnath and Rawat 1976; Kedharnath *et al* 1976).

3.7 *Poplars: (Populus spp.)*

Poplars, particularly clones of *Populus deltoides*, have a good future as a plantation crop in certain regions of North India. The clones so far tried are those that have been tested and selected for site and climatic conditions obtaining abroad. Testing of some of these exotic clones in the hope of identifying some amongst them as suitable for us is certainly a useful short cut approach to get something without much investment. But a more logical and realistic approach would be to develop our own clones of the promising exotic species *P. deltoides.* An approach currently being taken up in Uttar Pradesh envisages (i) collection of seed resulting from open pollination on some of the good female clones, (ii) attempt at controlled hybridisation between selected female and male clones should they exhibit synchronised flowering. It is proposed to raise seedlings from the seeds resulting from the above two approaches and test them in the appropriate region and then the promising plants from amongst these will be cloned. Tests for resistance to important diseases and insect pests will also be carried out.

While this work with exotic clones of *P. deltoides* progresses, it has also been planned to work on the genetic upgrading of *P. ciliata* and *P. gamblei*, two of our native poplars. A programme of genetic improvement work has been proposed for the poplars in India by Kedharnath (1979). Khurana and Khosla (1982) have been active in the selection of desirable phenotypes in *P. ciliata* and studying their variation in provenance testing. Khosla *et al* (1979) have also assessed the sex ratio in natural population of this species and studied the correlation between the sex of the tree and its growth.

4. Strategies for the future

Problems of immediate importance and finding solutions to them certainly deserve high priority and in this context tree breeding programmes had set high priority for the selection of plus trees and assembling them in clonal seed orchards so that as soon as the

orchards started producing regular crops of seeds, genetically improved planting material become available for raising the new plantations. That would mean immediate gains. But it is also necessary tò think of the longterm goal and plan for building material for advance generation or multigeneration breeding programmes. In developed countries where forest tree breeding programmes have been in operation for a long time tree breeders have given much thought in this direction. For example, Bourdon *et al* (1977) examined a wide range of alternative mating designs for various purposes including estimates of variances and combining abilities, development of breed populations and production of seed. They found that no single design was best for all purposes and no single purpose will be served by only one design. According to Lindgren (1977) reasonably good progeny tests can be made with a limited number of trees in any of the several designs including common testers, partial diallels, polycrossers and pollinations in seed orchards.

Strategies suggested for the development of long-term genetic improvement programmes by different experts in the field of tree breeding differ quite markedly. However, they all agree on the need to separate the short-term function of seed production from the long-term goal of developing and maintaining broad-based genetic populations for future advances in the tree improvement.

The commonest example of a production population is a seed orchard. In the orchard we generally tend to increase the genetic gain by increasing the selection differential. This may appear as a conflict to maintaining a broader genetic base for future breeding work. In the past there has been rigorous selection of plus trees so that only the best or more outstanding individuals were included in the seed orchard. However, the present tendency is to select a large number of good trees (rather than a few super trees) in the first round of selection of plus trees. Thus it is expected that it would not only ensure a broad genetic base than before but would also facilitate a reasonable level of improvement in the second and subsequent generations (Pederick and Griffin 1977). It is very satisfying to know that today amongst foresters there is an increasing appreciation of the role and potentiality of genetics and tree breeding in maximising production from the forest plantations.

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Some unusual features in the embryology of Angiosperms

B M JOHRI and K B AMBEGAOKAR
Department of Botany, University of Delhi, Delhi 110 007, India.

Abstract. Attention has been drawn to selected examples of unusual features in the embryology of angiosperms. The study of reproductive processes through microcinematography reveals sequential stages in living material which add a new dimension to our investigations. The unusually elongated embryo sacs in *Moquiniella*, synergid haustoria in *Cortaderia* and *Quinchamalium*, aggressive invasion by chalazal endosperm haustorium in the pedicel in *Olax* and *Opilia*, zygotic mantle in *Acrotrema*, highly polyploid suspensor cells in *Phaseolus*, undifferentiated embryo in *Eriocaulon*, and integumentary embryos in Compositae and Orchidaceae are briefly discussed. The association of bacteria with the flower and seed of *Ardisia* is essential for its perfect growth. Angiocarpy in *Tambourissa*, intracarpellary pollen grains in *Butomopsis*, and free-nuclear proembryo in *Paeonia* are typical gymnospermous characters in confirmed angiosperms.

Keywords. Angiocarpy; coenocytic zygote; endosperm; hyperstigma; suspensor.

1. Introduction

The foundation of our knowledge of embryology was laid toward the middle of the 19th century. By 1900 most of the basic facts had been discovered (see Maheshwari 1950). During the last 50 years, several taxa have shown unusual features, and some examples are cited here: (i) study of embryology through microcinematography, (ii) unusually long embryo sac, (iii) synergid haustoria, (iv) synergid and antipodal haustoria, (v) endosperm haustoria, (vi) zygotic mantle, (vii) suspensor haustoria, (viii) polyploidy in suspensor (ix) organless embryos, (x) integumentary embryo, (xi) association of bacteria with flower and seed, (xii) angiocarpy, (xiii) pollen grains in the stylar canal and ovary, and (xiv) free-nuclear embryo.

2. Microcinematography

In *Jasione montana* the ovules have a thin integument, and are more or less transparent. Erdelská (1969, 1982) made a microcinematographic study of living ovules and described the movement of the organelles in the central cell of the mature embryo sac, and the development of endosperm and embryo. The contraction cycle of nucleolar vacuole in the secondary nucleus ranges from 7 min to several hr. The contraction takes about 20 sec. The mitotic cycle during earlier stages of endosperm is relatively short, (6–8 hr).

There is a well developed endothelium at the mature embryo sac, and during early stages of development of endosperm. The endosperm is of the cellular type. The primary endosperm nucleus divides transversely followed by another transverse division in the micropylar chamber and a vertical division in the chalazal chamber. The rhythm of development of endosperm could be studied only up to the 8-celled stage since, later, the transparency of the material decreases.

The sequence of development and parameters of the linear phase of zygote were critically analysed (microcinematographically). The zygote elongates (60–80 μm at 5–6 $\mu m\,hr^{-1}$) (Erdelská and Klasová 1978) and the linear phase of the embryo development lasts about 36 hr; later, the thickness of the developing endosperm masks the embryo.

Thus, microcinematography provides a new approach to study living 'transparent' material. The streaming of cytoplasm, movements of organelles and nuclei, nuclear division and laying down of cell-plates can all be clearly visualized. A full length cinematographic film is now commercially available, and makes the understanding of reproductive processes very interesting and simplified.

3. Unusually long embryo sac

Moquiniella rubra (Johri and Raj 1969) shows the longest Polygonum type of 8-nucleate embryo sac in Loranthaceae. Four to nine embryo sacs develop concurrently. At the 4-nucleate stage, the tip of the embryo sac extends into the style and stigma. Subsequently, the tip (with the egg apparatus, zygote or the bicelled proembryo) curves backward. Thus, the orientation of the tip becomes hooked. At the mature embryo sac stage the antipodal cells are left *in situ* and the chalazal end extends up to the hypostase. The length of the straight arm of the embryo sac is 44 mm, whereas the backwardly curved portion is 4 mm, i.e., a total length of 48 mm (figure 1). The length of the embryo sacs in Loranthaceae varies from 16 mm in *Helicanthes elastica* (Johri *et al* 1957) to 42 mm in *Tapinostemma acaciae* (Johri and Prakash 1965).

The presence of the embryo sac in the style and stigma is itself so unusual, and the backward curvature of the tip of embryo sac adds a further novel feature.

The polar nuclei fuse below the egg-apparatus. The primary endosperm nucleus moves to the lower part of the embryo sac (in the ovary) where composite endosperm is produced.

4. Synergid haustoria

Cortaderia jubata (Philipson 1978) is a non-pseudogamous apomict, where pollen grains are not produced in the anthers. The ovule is bitegmic and tenuinucellate; the megaspore mother cell degenerates early. Two to three nucellar cells, in the chalazal region, develop into aposporous embryo sacs. The embryo sac is usually 6-nucleate—two antipodals which divide early to form a crescent-shaped tissue, two polar nuclei, one synergid, and one egg. Embryo sacs with 3–8 nuclei have also been observed. The mature gametophyte shows a well developed synergid haustorium (the other synergid collapses) which penetrates the micropyle, and extends between the outer integument and ovary wall (figure 2A). Even when there are more than two aposporous embryo sacs, only two synergid haustoria develop. During earlier stages the active synergid haustorium shows conspicuous wall ingrowths (*cf.* transfer cells) and granular cytoplasm. As the activity of the haustorium declines, its cytoplasm becomes less homogeneous concurrently with the development of endosperm. The remnants of the haustorium persist up to the globular stage of the proembryo. Rarely, the egg also develops a haustorium which extends beyond the micropyle and abbuts against the ovary wall. The synergid and egg haustoria need to be reinvestigated.

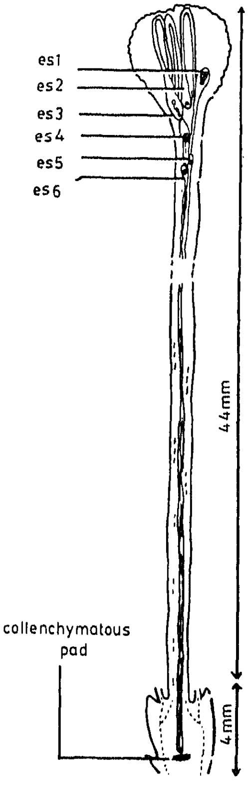

Figure 1. *Moquiniella rubra* (diagrammatic), longisection of gynoecium with six (es_1–es_6) embryo sacs; three embryo sacs (es_2, es_3, es_4) have reached up to the stigma and curved backward (after Johri and Raj 1969).

The central cell contains one or two polar nuclei. The inner surface of the central cell is covered by transfer cell ingrowths. The laterally placed 4–5 antipodal cells also show transfer cell ingrowths, confined only to the outer wall.

The three aposporous embryo sacs in an ovule may be at three different stages of development. In figure 2B es_1 is at the mature embryo sac stage, es_2 shows an inversely-oriented globular proembryo and a group of antipodal cells, and es_3 probably has reverse polarity with only a group of antipodal cells at the micropylar end.

The development of endosperm is autonomous (without triple fusion), and of the Nuclear type; the nuclei are peripherally disposed. Later, wall formation takes place, and an aleurone layer differentiates while the rest of the endosperm remains starchy. The endosperm may also develop in the absence of the embryo.

The development of the egg is parthenogenetic, and it usually divides after the formation of free-nuclear endosperm. The segmentation pattern of the parthenogenetic embryo is irregular. The micropylar cell remains connected with the embryo sac wall, and appears to function as a suspensor cell. The mature embryo is comparable to that of the Gramineae (Tribe Arundineae). In twin aposporous (nucellar) organized sacs, two embryos mature in the seed and germinate.

An interesting feature is that the embryos in diploid embryo sacs are at the globular stage when the inflorescence emerges from the upper leaf-sheath. Secondly, all other non-pseudogamous grasses show gonial apospory or diplospory; *C. jubata* exhibits somatic apospory.

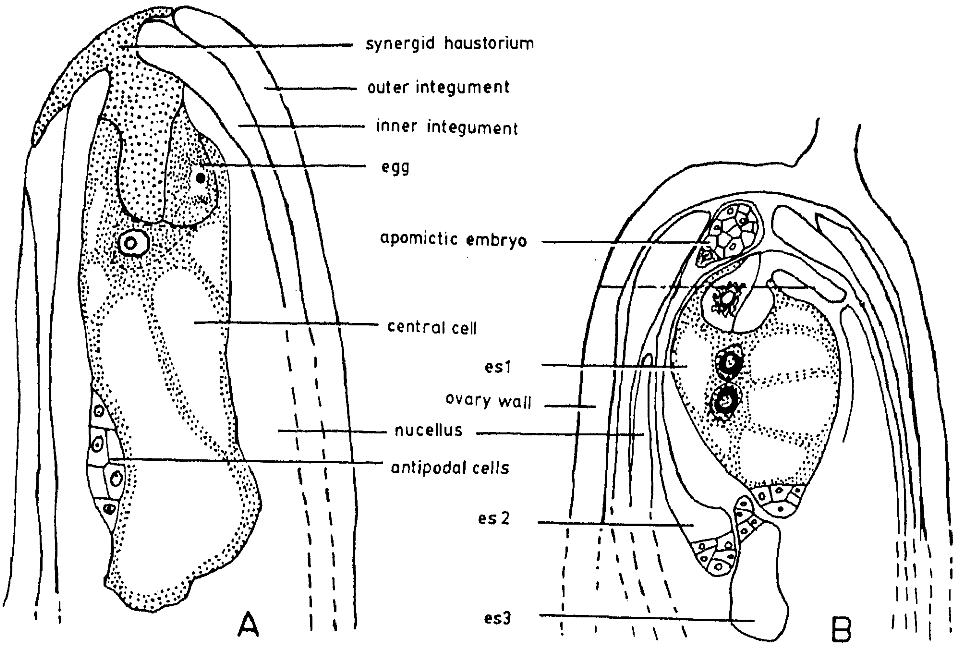

Figure 2. *Cortaderia jubata.* **A.** Longisection of upper part of ovule with egg, synergid haustorium in the micropyle, central cell, and laterally situated antipodal cells. **B.** Longisection of ovary; the ovule shows three aposporous (nucellar) embryo sacs. Embryo sac es_1 with egg, synergid haustorium, two polar nuclei, and antipodals; es_2 contains an inverted (?) globular proembryo and antipodals; es_3 is in reverse polarity (?) with antipodals at micropylar end (after Philipson 1978).

5. Synergid and antipodal haustoria

Synergid haustoria are common in Compositae, and antipodal haustoria in Rubiaceae.

The condition in *Quinchamalium chilense* (Santalaceae) (Agarwal 1962) is most unusual. At the 4-nucleate stage of the embryo sac, the tips of both the synergids extend up to the base of the ovary, then along the vascular supply of the style and, finally, reach up to one-third the length of the style.

The antipodal nuclei do not organize into independent cells so that there is an antipodal chamber with three nuclei. The tip of the antipodal chamber elongates, passes through the funiculus, reaches the placenta, and branches therein. The antipodal nuclei become hypertrophied and, sometimes, 5–7 nuclei are delimited by a partition wall.

A dissected embryo sac with synergid and antipodal haustoria is shown in figure 3. Such an extensive development of synergid and antipodal haustoria is unique in angiosperms.

6. Endosperm haustoria

Endosperm haustoria are quite common in the Polypetalae, Sympetalae and monocots, and in Nuclear, Helobial and Cellular types of endosperm. The families

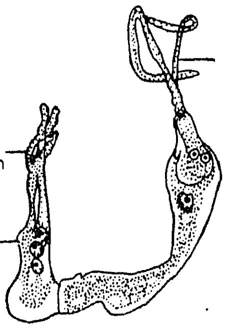

Figure 3. *Quinchamalium chilense*, embryo sac with well developed synergid and antipodal haustoria (after Agarwal 1962).

Olacaceae and Opiliaceae (Santalales) exhibit extragynoecial development of the chalazal endosperm haustorium.

6.1 *Olacaceae*

In *Olax stricta* (Kuijt 1969) and *O. wightiana* (Agarwal 1963) the female gametophyte elongates and its tip grows beyond the ovule into the base of the stylar canal. A lateral caecum develops close to the chalazal end, and extends in the basal region of funiculus.

The primary endosperm nucleus divides below the level of caecum, followed by a wall which delimits the chalazal and micropylar chamber. The chalazal chamber functions as an aggressive haustorium, becomes 2–4-nucleate, and the nuclei undergo hypertrophy and polyploidy. The haustorium extends through the base of the ovule into the base of the ovarian tissue. After reaching the ovary the tip of the haustorium branches, and some of the branches invade the pedicel (figure 4A). The remnants of the chalazal haustorium persist in mature seeds.

6.2 *Opiliaceae*

In *Opilia amentacea* (Swamy and Dayanand Rao 1963) the division of the primary endosperm nucleus leads to the formation of a smaller micropylar and a long tubular chalazal chamber. The micropylar chamber undergoes repeated divisions and forms the bulk of endosperm tissue. The chalazal chamber, with centrally located nucleus, extends towards the thalamus (figure 4B). In dissections of living material, the cytoplasm exhibits streaming movement and the direction of flow changes frequently. With subsequent growth of the endosperm, the chalazal chamber (haustorium) penetrates deeper into the axial region of the pedicel (figures 4C, D).

The length of the haustorium is 820–950 to 1151–1200 μm (from the point of origin to the point of entry into the pedicel), and 200–260 to 300–320 μm of the haustorium lies in the pedicel. In the pedicel the haustorium grows axially, and intercellularly between the thin-walled cells of the pith.

The development of the endosperm in *Aeginetia indica* (Tiagi 1952) is of the Cellular type. The primary endosperm nucleus divides transversely separating a chalazal chamber from the micropylar chamber. The chalazal chamber directly functions as a weak haustorium which later becomes compressed.

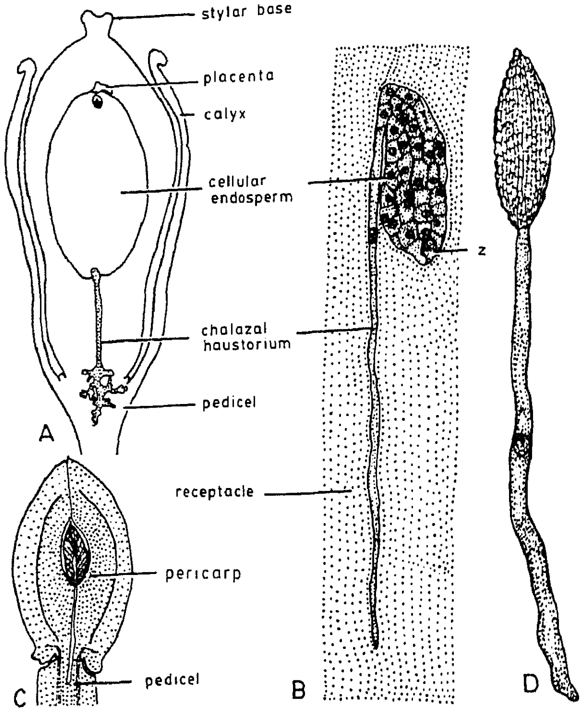

Figure 4. **A.** *Olax stricta*, longisection of young fruit; the chalazal endosperm haustorium penetrates as far as the pedicel (after Kuijt 1969). **B–D.** *Opilia amentacea*. **B.** Central region of ovule (LS) with zygote, cellular endosperm, and chalazal endosperm haustorium reaching up to the receptacle. **C.** Longisection of fruit; the chalazal endosperm haustorium has penetrated up to the pedicel. The outer pericarp is parenchymatous and the inner sclerenchymatous. **D.** Cellular endosperm and associated chalazal endosperm haustorium (dissected from **C**) (after Swamy and Dayanand Rao 1963).

The micropylar chamber undergoes a vertical division, followed by a transverse division. The middle tier divides repeatedly giving rise to endosperm proper. The nuclei of the haustorial cells undergo hypertrophy. The 2-celled micropylar haustorium extends around the seed coat, gives out hypha-like branches which digest the contents of the testa. The extensions of the haustorium penetrate the epidermis of the testa and hang out forming a fringe. When the minute dried seeds are soaked in water, the testa appears covered by 'fur'—the remnants of haustorial extensions. Such a course of events is not reported in any other taxon.

7. Zygotic mantle

Swamy and Periasamy (1955) reported the development of a zygotic mantle in *Acrotrema arnottianum*. " . . . the mantle is a modification of the zygotic membrane itself (the so-called wall of zygote)". The zygotic membrane is tenuous. Further " . . . the zygotic wall is not a true wall and is not laid down as a result of phragmoplastic behaviour during gametogenesis. The physical nature of such a wall

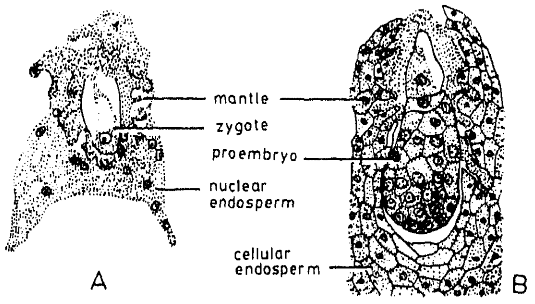

Figure 5. *Acrotrema arnottianum.* **A.** Zygote with 'mantle', and nuclear endosperm. **B.** Globular proembryo, the micropylar region has emerged out of the mantle; note cellular endosperm (after Swamy and Periasamy 1955).

may be compared to a thickened plasma membrane which is plastic and is capable of undergoing reversible structural changes during development".

Free-nuclear divisions occur in the endosperm while the protoplast of the zygote shrinks, and a space appears between the protoplast and the membrane. As the zygote elongates, the outer surface of the membrane shows weak undulations. In later stages the membrane differentiates into two zones: the (a) inner zone becomes gelatinous and lamellated, while the (b) outer zone is densely cytoplasmic (figure 5A).

By the time the zygote divides to form a young globular proembryo, the mantle develops finger-like finely-vacuolate protrusions.

The early proembryo pushes out of the zygotic mantle, except the micropylar region which remains embedded in the mantle (figure 5B).

As wall formation occurs in the endosperm, the mantle develops numerous coralloid protuberances which penetrate the intercellular spaces of the cellular endosperm.

With the accumulation of lipoid and other inclusions, the protuberances in the endosperm wither. Their remnants persist in the mature endosperm and appear as radiating streaks.

8. Suspensor haustoria

Suspensor haustoria are quite common in embryos of several taxa: Cuscutaceae, Leguminosae, Podostemaceae and Tropaeolaceae.

The embryogeny in *Cuscuta reflexa* (Johri and Tiagi 1952) shows several noteworthy features. By a transverse division the zygote produces an apical (a) and a basal (b) cell. The apical cell and its derivatives undergo repeated transverse divisions to produce a uniseriate row of seven tiers (a_1–a_7). Of these, five tiers (a_1–a_5) produce the embryo proper, while the upper two tiers (a_6 and a_7) contribute to the suspensor.

a_6 divides longitudinally and transversely giving rise to two tiers of cells which never become vesicular and multinucleate, but form a neck which protrudes into the adjacent coenocytic derivatives of a_7.

a_7 may remain undivided, or divide transversely or longitudinally. Except for

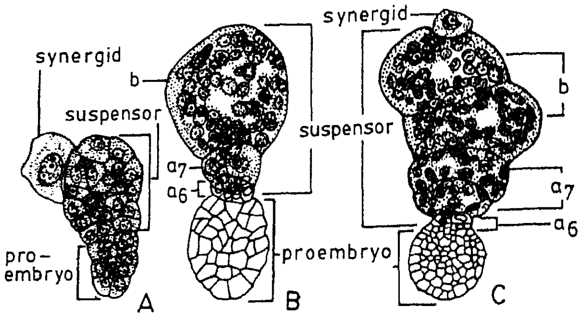

Figure 6. *Cuscuta reflexa.* **A.** Proembryo, suspensor comprises uninucleate cells; note the absence of vesicular coenocytic suspensor cells. **B.** Basal cell *b*, coenocytic and haustorial suspensor, at globular proembryo. **C.** Four-celled suspensor of coenocytic and vesicular cells, at globular proembryo. Note the persistent synergid in **A** and **C** (after Johri and Tiagi 1952).

embryos with non-vesicular suspensor, a_7 and its daughter cells become vesicular, vacuolate and coenocytic.

The basal cell (*b*) contributes to the suspensor, and follows the same pattern of divisions as a_7. The daughter cells of *b* may remain uninucleate and non-vesicular (figure 6A). Mostly the basal cell (*b*) remains undivided, but becomes vesicular, vacuolate and multinucleate (figure 6B). The maximum number of nuclei in a cell may be up to 70 but, after fusion, the number may be reduced. Such nuclei are multinucleolate and polyploid. Occasionally, the basal cell divides longitudinally, or transversely (figure 6C), and the derivative cells become vesicular and coenocytic.

Thus, the haustorial, coenocytic suspensor cells originate from the derivatives of a_7 and basal cell (*b*). There is no doubt that the suspensor functions as an aggressive haustorium. Such a feature is not known in any other angiosperm.

The mature embryo lacks radicle, and is a coiled structure which is hypocotyledonary in nature. The shoot apex differentiates at the narrow tip and has a few rudimentary scale leaves. The remnants of one synergid and suspensor persist until late stages of development of the embryo.

9. Polyploidy in suspensor

Polyploidy in suspensor cells is quite common. The most studied taxon is *Phaseolus*.

In *Phaseolus vulgaris* (Souèges 1950) the basal cell of the 2-celled proembryo produces a multicellular suspensor with uniform, uninucleate, giant cells.

In *P. coccineus* (Yeung and Clutter 1979), at the proembryo stage, the suspensor cells form wall ingrowths (transfer cells) which become very conspicuous at the heart-shaped stage. These transfer cells are involved in transport of nutrients to the developing embryo. The organelles such as mitochondria, plastids and endoplasmic reticulum continue to increase in the cells and, at the late globular stage, the cells become packed with them. The nuclei give a lobed appearance and contain micronucleoli.

Due to endoreduplication the nuclei exhibit high polyploidy (Nagl 1974):

Species	Ploidy (*n*)
Phaseolus vulgaris	2048
P. acutifolius	1024
P. coccineus	8192
P. hysterinus	4096
P. mungo	512
P. lunatus	256
P. tuberosus	256

Cionini *et al* (1976) have shown that gibberellic acid (GA_3) (10^{-8} to 10^{-6}M) can replace the function of suspensor of the heart-shaped and early cotyledonary embryos (length 0·5 to 1·5 mm).

In later stages (length 2 to 3 mm) GA_3 inhibits the development of suspensor-deprived embryos. The suspensor, during heart or early cotyledonary stages, can synthesize growth regulators necessary for the growth of the embryo.

In *P. vulgaris* (Yeung 1980) when labelled ^{14}C-sucrose was administered to the pods (embryos in seeds at heart-shaped stage), after 3 hr the suspensor contained the maximal amount of label as compared to the other parts of the embryo. The uptake ratio (% counts/% fresh weight) was 4·49 in suspensor, 0·88 in the portion of embryo attached to the suspensor, and 0·58 in cotyledonary region. Therefore, "the suspensor of the late heart-stage embryo is the major site of nutrient uptake". During mid-maturation stages, the activity in the suspensor cells slows down but increases in the cotyledonary region.

The giant suspensor cells of *P. coccineus* (Nagl 1977), at the late heart-shaped stage, show transformation of the filiform leucoplasts into cytosome-like structures which show acid phosphatase activity. These are termed 'plastolysome' which causes the suspensor cells to undergo autolysis.

10. Organless embryos

In a number of taxa the mature embryo is devoid of organs, but is nevertheless differentiated, *e.g.* in Orobanchaceae and Orchidaceae. The embryogeny in Eriocaulaceae has been investigated by Ramaswamy and Arekal (1982a, b). The mature embryo, in the seed, lacks the usual organs.

The spherical zygote (figure 7A) undergoes a transverse division to form the cells *ca* and *cb* (figure 7B), in *Eriocaulon xeranthemum* (Ramaswamy and Arekal 1982a), *Leiothrix nubigena*, *Paepalanthus bifidus* and *Syngonanthus nitens* (Ramaswamy and Arekal 1982b). A vertical division first in *ca*, and then in *cb* forms a 4-celled proembryo (figure 7C). Periclinal division occurs in each cell; anticlinal divisions in the outer four cells differentiate a protoderm which delimits a group of four cells in the centre (figure 7D).

The two adjacent derivatives of *ca* are the initials of epicotyl and cotyledon (figure 7E). The divisions in the epicotyledonary zone form only 2–4 cells, whereas repeated divisions occur rapidly in the cotyledonary zone and its subjacent cell tiers so that the cotyledonary sector of the embryo becomes fan-shaped (figure 7F). Further development of the cotyledon occurs during germination of the seed.

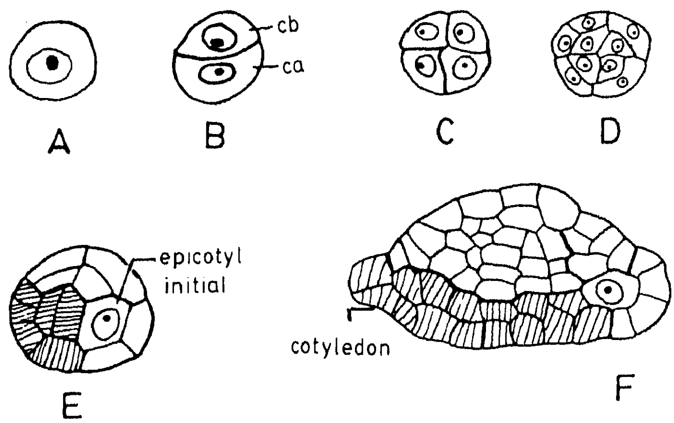

Figure 7. *Eriocaulon xeranthemum.* **A.** Zygote. **B.** Two-celled proembryo. **C.** Four-celled proembryo. **D.** Embryo with protoderm demarcated. **E.** Globular proembryo with epicotyl initial and cotyledonary initials (*striped cells*); organogenesis does not occur. **F.** Immature embryo with epicotyledonary initial (*nucleated cell*) and cotyledon (after Ramaswamy and Arekal 1982a).

The basal cell contributes two rows of cells at the radicular pole but, in a mature seed, a radicle fails to organize.

Ramaswamy and Arekal (1982b) state: "The cotyledonary and epicotyledonary loci share equal amounts of the embryonic shoot apex as in most monocotyledons. The cotyledon and its adjacent epicotyl are both terminal on the embryonic axis".

11. Integumentary embryos

Somatic embryos of integumentary origin are not very common. Adventive embryos develop from the inner integument in Orchidaceae, and from endothelium in Compositae.

11.1 *Compositae*

In *Melampyrum divaricatum* (Maheswari Devi and Pullaiah 1976) 54 % of the ovules in a head show embryo sacs with degenerated egg and secondary nucleus, and six or seven cells of the integumentary tapetum enlarge and become richly cytoplasmic. These cells divide transversely followed by further transverse and longitudinal divisions resulting in different stages of proembryos. The authors point out that the sequence of divisions is identical to the development of the zygotic embryo which is doubtful. Such proembryos are devoid of suspensor. They project into the embryo sac, and only one of them develops into a typical dicotyledonous embryo.

Of 60–70 % of the ovules which develop into seeds, only 10–15 % contain zygotic embryo. The rest of the seeds probably have embryos derived from endothelial cells.

In *Carthamus tinctorius* (Maheswari Devi and Pullaiah 1977) 8–9 endothelial cells develop into adventive embryos, but only 1–2 reach up to the heart-shaped stage. The authors do not give any further details.

11.2 *Orchidaceae*

In asexual individuals of *Spiranthes cernua* (Swamy 1948) the 2-celled pollen develops normally, long before anthesis. But, by the time the flower opens, the pollen grains degenerate.

One female gametophyte develops only up to the 4-nucleate stage, and the size and position of nuclei vary so that the polarity is disturbed. Thus, the "female sexual cycle" is suppressed.

In the bitegmic ovules, the inner integument comprises 2–3 layers of cells. The cells of the inner epidermis individually, or in groups, undergo divisions and exhibit potentiality to develop into adventive embryos.

During meiotic divisions of megaspore mother cell the richly cytoplasmic integumentary cells, some have undergone division already, are conspicuous. The mature seed contains 2–6 adventive embryos. When the terminal cell of the integument develops into a somatic embryo, it flares out of the outer integument into the ovarian cavity and the undifferentiated embryo lies outside the ovule.

12. Association of bacteria with flower and seed

There are very few taxa which require the association of bacteria for their healthy growth.

Bacterium follicola is a symbiont associated with *Ardisia crispa* (De Jongh 1938). The bacteria are non-acid, gram negative rods with a tendency to become non-motile, with a mucilagenous membrane, and form bacteroids. They are active in meristems of stem, but remain inactive (with reversible capacity) in dormant buds, and can undergo several years of rest. They cannot fix nitrogen.

The bacteria adhere to the radicular end of the embryo. On germination of the seed the bacteria infect the axillary buds, and then the terminal buds. They occur in plants with active and dormant meristems (except root and anther meristem), and in the flower buds. They grow in inflorescences, floral parts of young and old flowers, carpels and, finally, lodge in the micropylar region of the integuments. In mature seed the colourless motile bacteria persist between embryo and endosperm.

The bacteria-free plant, the "Cripple", has a juvenile appearance. Presumably, the association of bacteria is necessary for the normal development of the plant body. Further investigations would be rewarding.

13. Angiocarpy

The unisexual flowers of *Tambourissa religiosa* (Endress 1980) are enclosed in urceolate cups (figure 8A). A pore at the apex of the thick floral cup leads a narrow channel to the inside. The perianth (tepals) is extremely reduced.

In male flowers the anthers are numerous and sessile. Each anther dehisces by a single slit from both sides over the apex. The secretion of mucilage is absent in male cups.

The carpels remain embedded in the thick-walled floral cup, and are connected by narrow channels to the centre of the cup. The carpellary surface is secretory. The female floral cups develop 'hyperstigma'. The epidermis of the tepals, and the cells

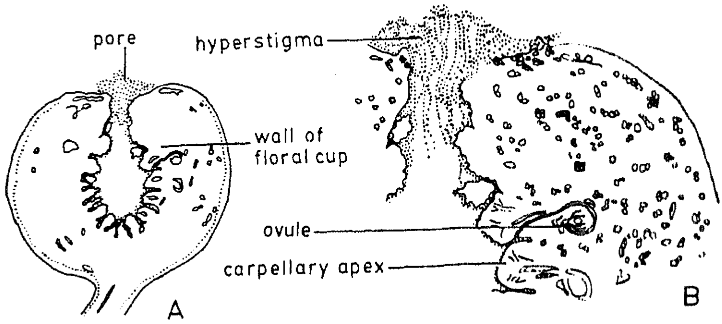

Figure 8. *Tambourissa religiosa*. **A.** Longisection of female floral cup with hyperstigma and carpels. **B.** Floral canal with hyperstigma and upper two carpels; *mucilage dotted, stone cells in groups* in fleshy wall of floral cup (after Endress 1980).

lining the central canal secrete mucilage which fills the centre of the cup. In *Tambourissa* the tissue around the hyperstigma decays, and produces a large quantity of mucilage.

At anthesis, mucilage oozes out of the pore of the female floral cup. The floral channel becomes wider and extends through the carpellary slits down to the micropyle of ovule, in each carpel (figure 8B). Thus, the mucilage which acts as a transmitting medium is continuous from the opening of the female floral cup to the carpels, and then to the micropyles of the ovules.

The pollination is entomophilous. The pollen grains are received at the hyperstigma which is extra-carpellary. There they germinate, and the pollen tubes pass to the ovules through the transmitting medium of mucilage.

Thus, there is a significant change in the site of reception and germination of pollen grains as compared to normal angiosperms. Endress (1980) terms this feature as 'angiocarpy', reflecting an intermediate behaviour between angiosperms and gymnosperms.

14. Pollen grains in the stylar canal and ovary

The pollen grains usually land on the stigma, germinate, the pollen tubes reach the ovary, enter the ovule, and bring about fertilization. Very unusual and unique features have been reported in a member of Alismaceae.

The occurrence of 5–8 three-celled pollen grains in the stylar canal of *Butomopsis lanceolata* (Johri 1936) is a rare feature and, according to Sahni and Johri (1936), "it can only be regarded as a relic of gymnospermy in a confirmed and unquestionable angiosperm".

The stylar canal secretes mucilage. The germinated pollen grain and portions of pollen tubes have been noticed in the stylar canal and, in one case, a pollen grain germinated directly on the surface of the ovule.

Sahni and Johri stated that such entry of pollen grains inside the stylar canal might be assumed due to "some sort of suction mechanism like that of the 'stigmatic drop' of gymnosperms, the stylar canal functioning like a micropyle".

Maheshwari (1960) compared this feature with that of *Ephedra* and *Gnetum*. In

Gnetum (Vasil 1959), sometimes, the pollen grains germinate in the stylar canal some distance away from the nucellus, or on the nucellus.

15. Free-nuclear proembryo

In most angiosperms the zygote divides by a transverse wall, in a few taxa by longitudinal wall. Both the cells usually contribute to the development of embryo; the contribution of each cell varies from plant to plant.

The development of embryo in *Paeonia anomala*, *P. moutan* and *P. wittmanniana* (Yakovlev and Yoffe 1957) and *P. lactiflora* (Yakovlev 1969) is "biphasic" and does not correspond to any of the existing angiosperms.

In the first phase the zygote enlarges, undergoes free-nuclear divisions, and becomes coenocytic (figures 9A, B). The nuclei lie peripherally, and centripetal wall formation commences between the nuclei, enclosing a cavity in the centre (figure 9C).

In the second phase meristematic zones of cells differentiate in the distal region. Repeated divisions occur and several embryonal buds develop (figure 9D). There may be as many as 25 meristematic zones, but their growth is arrested at different stages of development. Finally, one bud grows and differentiates into a typical dicotyledonous embryo (figure 9E).

Murgai (1959) claims that the zygote divides by a transverse wall as in other angiosperms, but the apical cell collapses and the development of the basal cell conforms to that described by Yakovlev and Yoffe (1957).

Cave *et al* (1961), Matthiesson (1962) and Moscov (1964) have supported Yakovlev's findings, and Murgai's observation should be regarded as erroneous.

The coenocytic phase in the embryogenesis of *Paeonia* is comparable to that in gymnosperms (*Ginkgo*).

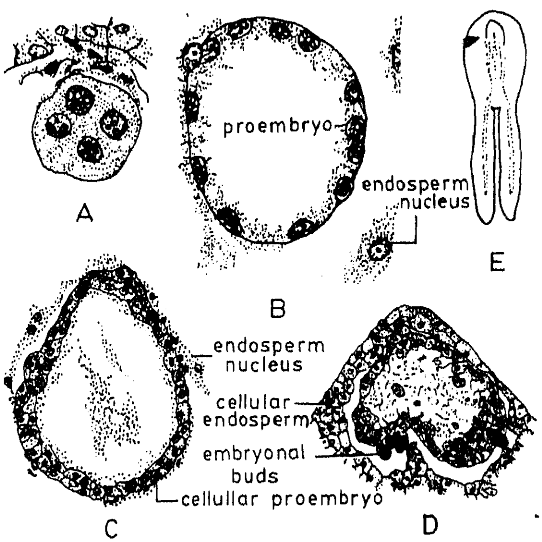

Figure 9. *Paeonia lactiflora*. **A, B.** Coenocytic proembryo. **C.** Proembryo with centripetal wall formation; there is a prominent central cavity. Note nuclear endosperm in **B** and **C**, **D.** Later stage; note embryonal buds and cellular endosperm. **E.** Dicotyledonous embryo (after Yakovlev 1969).

16. Conclusion

Of the numerous unusual features in the embryology of angiosperms, selected examples have been presented in the foregoing pages.

The association of bacteria in *Ardisia crispa* is responsible for healthy growth of plants. The role of bacteria should be investigated. 'Angiocarpy' in *Tambourissa religiosa*, presence of pollen grains of *Butomopsis lanceolata* in its stylar canal and in the ovary, and free-nuclear division in the zygote in *Paeonia* are all gymnospermous characters in typical angiosperms. These features, by no means, reflect any relationship between the two groups of seed plants, but may be considered as parallel evolution.

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Light and scanning electron microscopic study of seeds in *Nigella* L (Ranunculaceae)

BIR BAHADUR, S M FAROOQUI and K VIJAYA BHASKAR
Department of Botany, Kakatiya University, Warangal 506 009, India

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Abstract. Seed morphology of 6 species of *Nigella* L. (Ranunculaceae) viz., *N. sativa* L., *N. hispanica* L., *N. arvensis* L., *N. orientalis* L., *N. nigellastrum* (L) Willk. and *N. integrifolia* Regel, was studied utilising the light and scanning electron microscope to determine the significance of testa features as taxonomic characters. An artificial key based on spermoderm features is proposed to delimit the species studied. The present study supports the treatment of *N. integrifolia* Regel as a monotypic genus *Komaroffia integrifolia* (Regel) Periera.

Keywords. *Nigella* L; seed morphology; taxonomic significance; light microscopy; scanning electron microscopy.

1. Introduction

The genus *Nigella* L (Ranunculaceae) consists of 20 species indigenous to Mediterranean areas of Europe and Central Asia (Willis 1973). Various aspects of the genus *Nigella* have been examined by a number of workers, embryology (Kordyum 1957, 1959; Ly Thi Ba 1962; Vijayaraghavan and Marwah 1969 a, b), cytology (Strid 1965; Gillot 1970; Bhandari *et al* 1976; Suresh and Satyesh 1980), biosystematics (Strid 1970) palynology (Skavarla and Nowicke 1979). Recently Bouman (1978) discussed the seed and seed coat features (LM and SEM) of *Nigella damascena.* To date there is no comparative study on the microcharacters of the testa surface of the *Nigella* species presently studied. The application and use of SEM besides LM has helped in examining the surface features of species with small seeds. We have, therefore, examined 6 species of *Nigella* to assess the value of seed morphology (LM and SEM) by which the species may be distinguished and identified.

2. Materials and methods

Seed material of *Nigella sativa* L, *N. hispanica* L, *N. arvensis* L, *N. orientalis* L, *N. nigellastrum* (L) Willk and *N. integrifolia* Regel was obtained from Botanischer Garten, Der Universitat, Basel (Switzerland). Exomorphic characters like size, colour and testa pattern were observed under LM. For SEM study, the seeds were affixed on aluminium stub by transparent adhesive. The seeds were uniformly coated with gold following the sputtering technique (Damblon 1975), rotating at an angle of 45° to the vaporising filament at an accelerating potential of 10–15 kV and a part of the seed coat was uniformly photographed in all the species (using a Cambridge stereoscan microscope model S4-10 (England)).

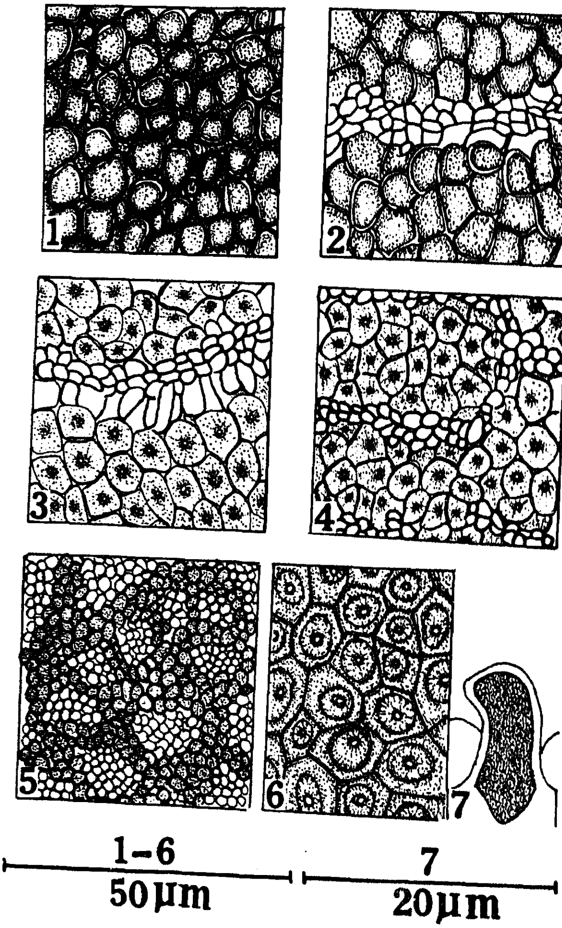
3. Observations

3.1 *Nigella sativa* L.

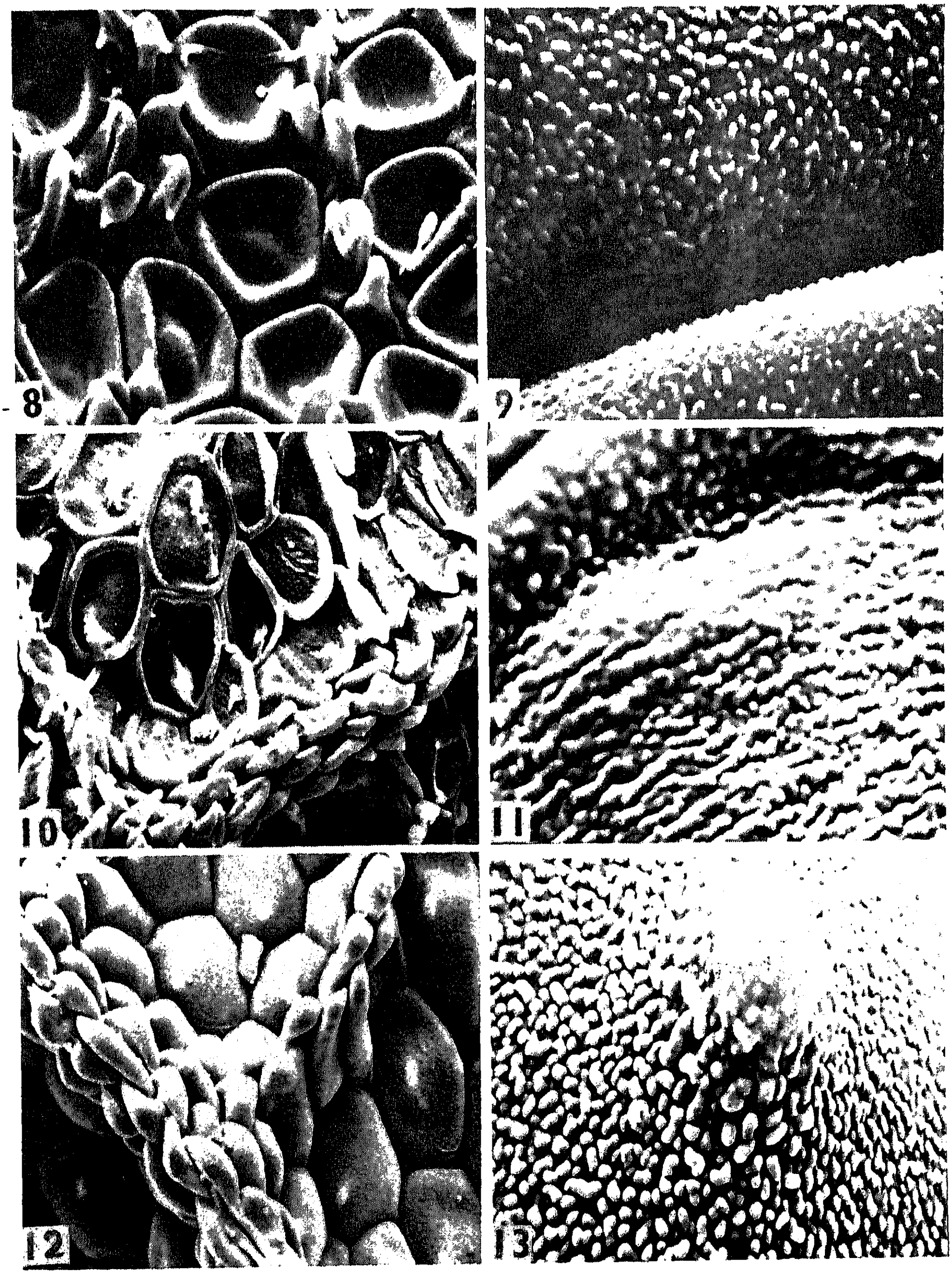
The mature seeds are $3{\cdot}25 \times 1{\cdot}8$ mm, flattened, triangular and black in colour. Testa (LM) shows a reticulate pattern of ridges. The epidermal cells between the ridges are tetragonal or circular in surface view (figure 1). Spermoderm (SEM) shows the epidermal cells with deep concavity looking like cupules and their surface is spinulose (figures 8, 9).

3.2 *Nigella hispanica* L.

The mature seeds are $2{\cdot}5 \times 1{\cdot}6$ mm, flattened, triangular and black in colour. Testa (LM) shows a reticulate pattern of ridges. The cells of elevated ridges are small, thick-walled and dark coloured. The epidermal cells between the ridges are comparatively



Figures 1–7. 1–6. Testa part of *Nigella sativa, N. hispanica, N. arvensis, N. orientalis, N. nigellastrum* and *N. integrifolia*. 7. Papilla of *N. integrifolia*.



Figures 8–13. SEM photograph of reticulate spermoderm of **8.** *N. sativa* (× 230), **9.** spinulose surface of epidermal cell (× 2300), **10.** *N. hispanica* (× 230), **11.** undulate surface of epidermal cell (× 2300), **12.** *N. arvensis* (× 230), **13.** nipple like projections and granulate surface of epidermal cell (× 2300).

large and polygonal in surface view (figure 2). Spermoderm (SEM) shows the ridges as small, elongated cells interwoven in chains. The epidermal cells between the ridges are large, polygonal with shallow undulate surface (figures 10, 11).

3.3 *Nigella arvensis L.*

The mature seeds are 2·4 × 1·5 mm flattened, ovate and black in colour. Testa (LM) shows the prominent elevated ridges composed of short, thick-walled and dark coloured cells. The epidermal cells between the ridges are comparatively large, pentagonal to hexagonal in surface view, with nipple-like projections (figure 3). Spermoderm (SEM) shows the elevated ridges as small, fusiform to spindle-shaped cells interwoven in chains with less prominent nipple-like projections on them. The epidermal cells between the ridges are flat, penta to hexagonal with distinct nipple-like projection, and of densely granulate surface (figures 12, 13).

3.4 *Nigella orientalis L*

The mature seeds are 3·8 × 1·8 mm flattened, globoid and black in colour. Testa (LM) shows a reticulate pattern of ridges. The cells of ridges are short-elongated, thick-walled and dark-coloured. The epidermal cells between the ridges are comparatively large, flat, penta to hexagonal in surface view with nipple-like projections (figure 4). Spermoderm (SEM) shows the elevated ridges as small, clavate-shaped compactly placed cells. The epidermal cells between the ridges are flat, penta to hexagonal with distinct nipple-like projections and of less granulate surface (figures 14, 15).

3.5 *Nigella nigellastrum (L) Willk.*

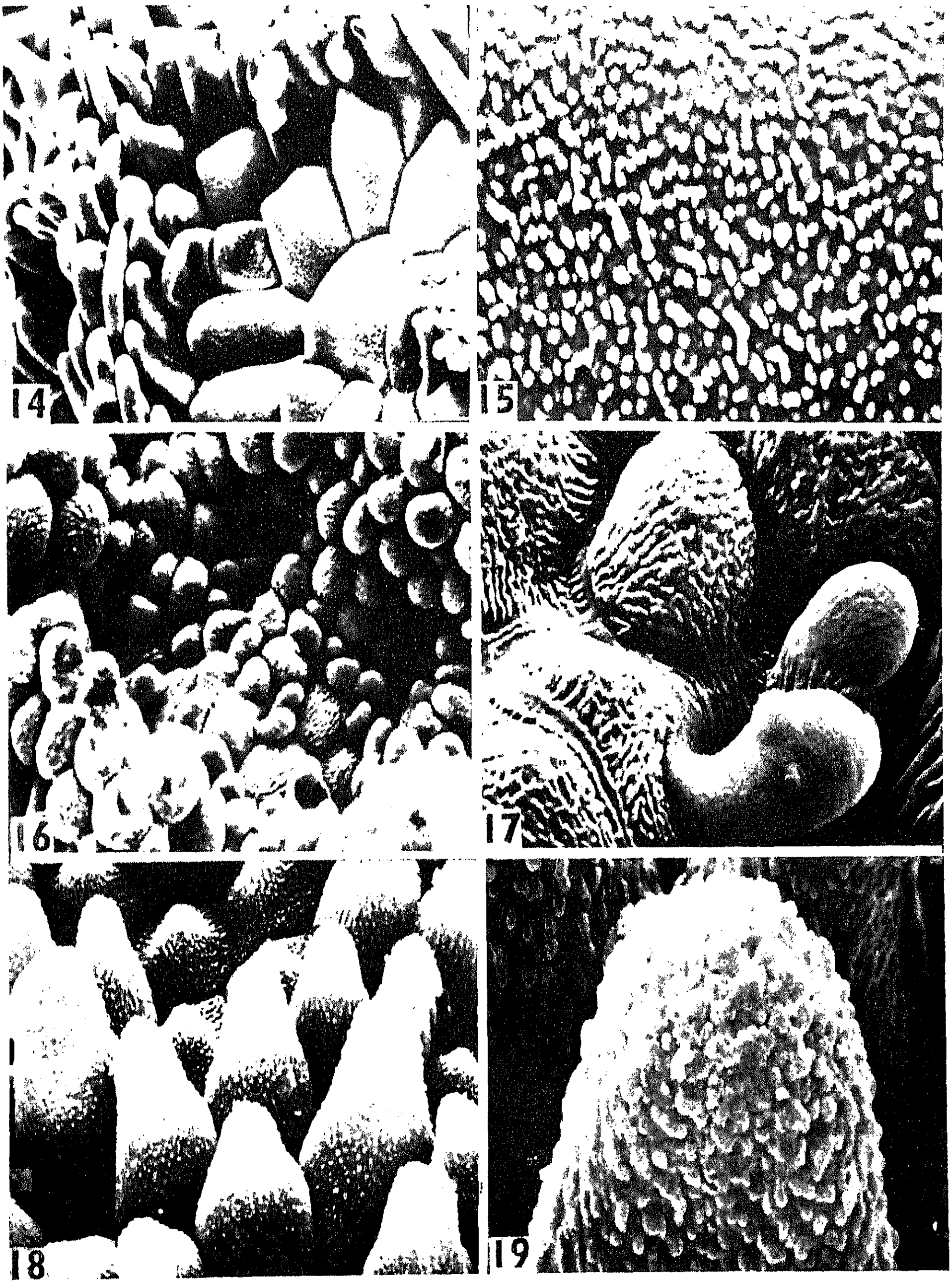
The mature seeds are 2·6 × 1·8 mm flattened, ovate and greyish black in colour. Testa (LM) shows a reticulate pattern of ridges and furrows. The cells of elevated ridges are large circular, thick-walled and dark-coloured. The epidermal cells between the ridges are short and circular in surface view (figure 5). Spermoderm (SEM) shows the elevated ridges of large, circular, reticulate cells, with few of them producing tomentose conical projections. The epidermal cells in furrows are polymorphic with secondary fine reticulations traversed with channel like net-work (figures 16, 17).

3.6 *Nigella integrifolia Regel.*

The mature seeds are 1 × 5 mm flattened, triangular and greyish in colour. Testa (LM) shows a glossy, papillate type, composed of penta to hexagonal cells projecting circular tips in surface views (figure 6). Spermoderm (SEM) shows numerous erected bottle like glossy papillae densely covered by warty structures (figures 18, 19).

4. Discussion

Mature seeds of *Nigella* species (1–3·8 mm in length) are black or greyish in colour. In general the seeds are flattened due to compact development within the capsules (Bouman 1978) and their outlines vary as ovate, globoid or triangular. The seed-coat



Figures 14–19. SEM photograph of reticulate spermoderm of **14.** *N. orientalis* (×230), **15.** granulate surface of epidermal cell (×2300), **16.** *N. nigellastrum* (×230), **17.** reticulate surface of epidermal cell with conical projection (×2300), **18.** SEM photograph of papillate spermoderm of *N. integrifolia* (×550), **19.** a papilla with warty surface (×2300).

surface (LM, SEM) in *N. sativa*, *N. hispanica*, *N. arvensis*, *N. orientalis* and *N. nigellastrum* is of more or less reticulate type, while *N. integrifolia* is of papillate type. The ridges are more prominent at the junction of flattened planes than the transversely oriented ridges of the integumentary part of the seed. The cells of ridges are small, elongated, compactly placed or in interwoven chains. The epidermal cells between the ridges are comparatively large, flat, circular to polygonal in surface view. Under the SEM, cells are cupule-like with a spinulose surface (*N. sativa*), shallow cells (*N. hispanica*), penta to hexagonal cells with prominent nipple like projections and granulate surface (*N. arvensis* and *N. orientalis*); cells polymorphic with fine secondary reticulations (*N. nigellastrum*). But the spermoderm of *N. integrifolia* is unique in possessing glossy bottle-shaped papillate outgrowths of warty surface. Skvarla and Nowicke (1979) similarly noted typical exine in pollen grains of *N. integrifolia* out of 8 species of *Nigella* studied. Further cytological studies by Strid (1970) also indicate variation in chromosomes number; *N. integrifolia* ($n = 7$) in contrast to other species ($n = 6$). Thus palynological, cytological and testa microcharacters support the treatment of *N. integrifolia* as a monotypic *Komaroffia integrifolia* (Regel) Lemos Periera. A key has been proposed on the basis of spermoderm features for the identification of various *Nigella* species studied.

Artificial key based on spermoderm features for the Nigella species studied

Seeds flattened, small, 2·4–3·8 mm, black.
  Testa reticulate:
    Epidermal cells cupulate, deep, spinulose surface .... *N. sativa*
    Epidermal cells polygonal, shallow, undulate surface .... *N. hispanica*
    Epidermal cells with nipple like projections, densely granulate surface, ridges cells spindle shaped interwoven in chains.......................... *N. arvensis*
    Epidermal cells with nipple like projections granulate surface, ridges cells clavate shaped and compactly placed .... *N. orientalis*
    Epidermal cells polymorphic, reticulate surface and with rare conical projections .... *N. nigellastrum*
Seeds flattened, small, 1·00 mm, greyish.
  Testa papillate:
    Epidermal cells bottle shaped glossy papillae of warty surface. . .*N. integrifolia*

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A contribution to the embryology of *Cicerbita alpina* (Linn.) Wallr.

T PULLAIAH and P SWARAJYA LAKSHMI

Department of Bio-Sciences, Sri Krishnadevaraya University, Anantapur 515 003, India

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Abstract. Development and structure of male and female gametophytes and fertilisation of *Cicerbita alpina* (Linn.) Wallr. have been studied.

Keywords. Embryology; *Cicerbita alpina.*

1. Introduction

Embryological studies in the family Compositae are quite extensive and date back to the previous century (Pullaiah 1984). Although many papers have appeared on the embryology of the family only 15 % of the species have been investigated embryologically. The genus *Cicerbita* includes 18 species which are chiefly distributed in the north temperate regions especially in the mountains (Willis 1973). In this paper *Cicerbita* has been investigated embryologically.

2. Material and methods

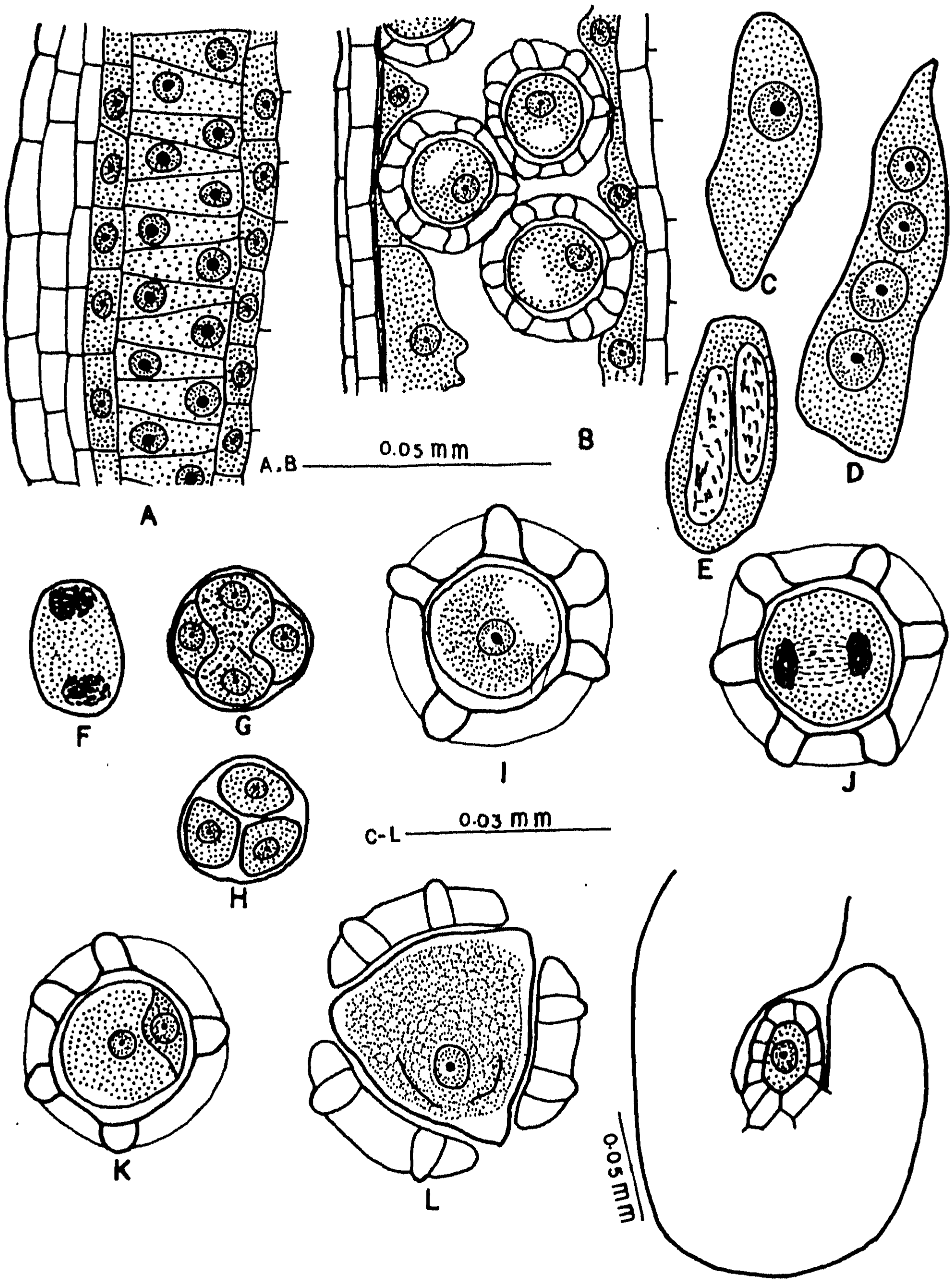
The material was collected from the Botanical Gardens of Moscow State University, USSR. Capitula at different stages of development were fixed in formalin-acetic-alcohol. Pieces of capitula were dehydrated in tertiary butyl alcohol series. Embedding was done in paraffin (m.p. 58–60°C). Microtome sections 8–10 μm thickness were cut and stained in Delafield's haematoxylin.

3. Observations

3.1 *Microsporangium*

The anther is tetrasporangiate. The youngest anther showed a single row of microspore mother cells in each anther lobe surrounded by the epidermis, endothecium, middle layer and single layered periplasmodial tapetum (figure 1A). The epidermal cells undergo anticlinal divisions and keep pace with the enlarging anther. Before dehiscence the endothecial cells elongate radially and develop thickenings on the inner and radial walls and become fibrous.

To begin with, the tapetal cells are uninucleate (figure 1C), but during subsequent development, they enlarge, the nuclei divide and the cells become bi- and tetranucleate (figure 1D). Fusion of these nuclei results in varying degrees of polyploidy. In some cases the four nuclei enter another mitotic division but the spindles fuse at the end of the division and two large polyploid nuclei are formed (figure 1E). The walls of the tapetal cells break down at the 1-nucleate pollen grain stage and the cytoplasm



Figures 1A–M. **A.** LS of anther lobe, wall layers and pollen mother cells; **B.** LS of anther lobe showing periplasmodium and one-nucleate pollen grains. **C–E.** Anther tapetal cells; **F.** Pollen mother cell in meiotic division; **G,H.** Decussate and tetrahedral pollen tetrads respectively. **I.** Uninucleate pollen grain; **J.** Pollen grain undergoing mitotic division; **K.** Two-nucleate pollen grain; **L.** Mature pollen grain; **M.** LS ovule, megaspore mother cell.

protrudes into the anther locule (figure 1B). The cytoplasm coalesces and forms the periplasmodium which is consumed by the developing pollen grains and no trace of it is left at maturity.

3.2 *Microsporogenesis and male gametophyte*

The pollen mother cells undergo meiotic divisions (figure 1F) resulting in tetrahedral (figure 1H) or decussate tetrads (figure 1G). Cytokinesis is simultaneous. The microspore enlarges, gradually becomes spherical and develops a thick exine and a thin intine. Due to the appearance of a large vacuole, the nucleus is displaced towards one side. The vacuole later disappears. The pollen grain procreates a small generative and a large vegetative cell, the former is closely appressed to the intine (figure 1J, K). Soon afterwards the generative cell gets pinched off and comes into the cytoplasm of the vegetative cell where it divides and forms two sperm cells. The sperm cells are filiform and much elongated. The pollen grains are 3-celled at the shedding stage with 3 germ pores (figure 1L).

3.3 *Ovary and ovule*

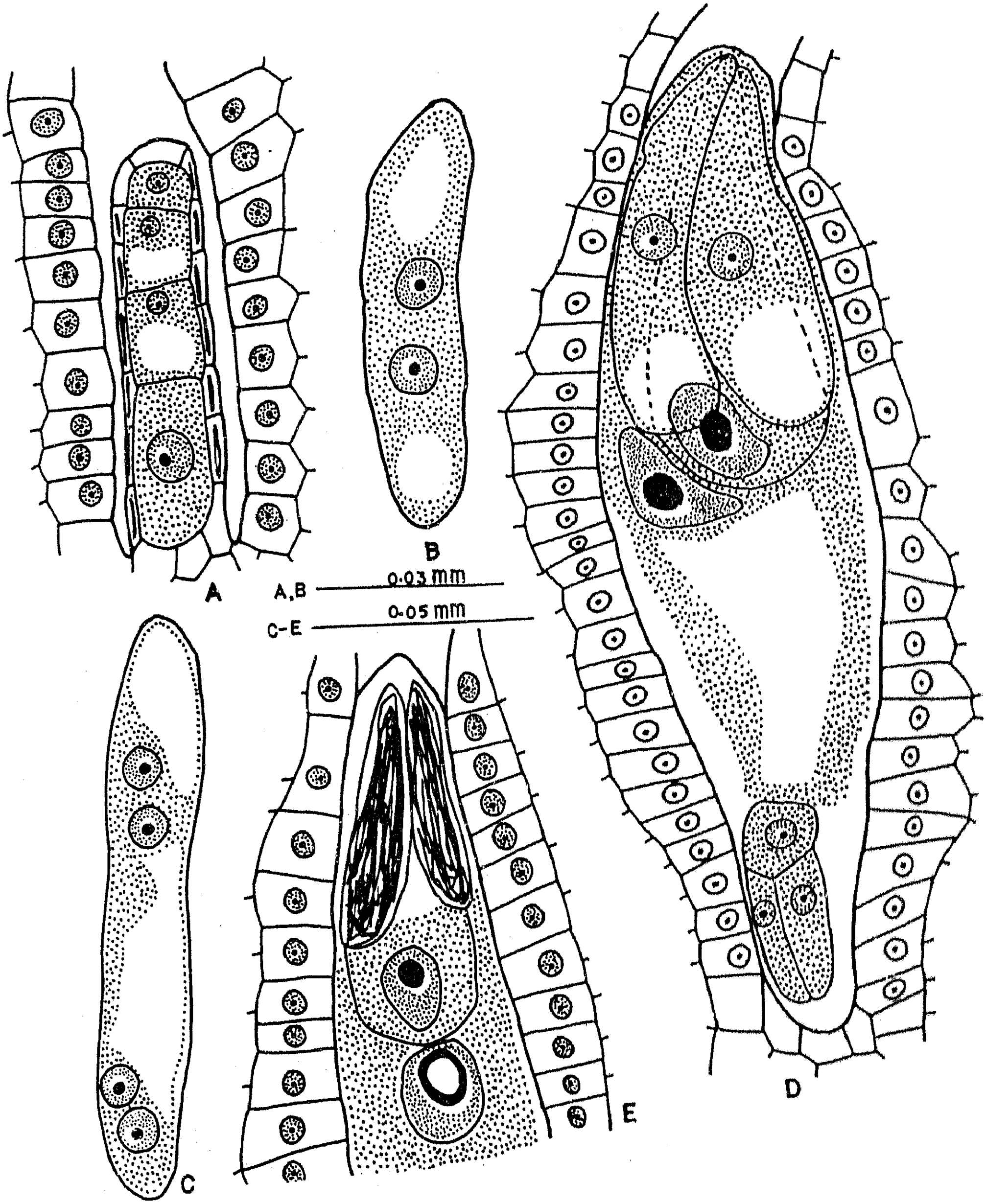
The ovary is inferior, bicarpellary syncarpous and unilocular with single basal ovule. The ovule arises as a small outgrowth at the base of the ovary but, during subsequent development, it curves and attains an anatropous condition at the megasporogenesis stage. The ovule is unitegmic and tenuinucellate (figure 1M). The innermost layer of the integument during megaspore tetrad formation differentiates into the integumentary tapetum (figure 2A). It remains uniseriate with uninucleate cells throughout its further growth (figure 2D). The epidermal cells lining the micropylar canal are elongated and appear glandular. These can be referred to as the obturatory cells. These cells probably guide the pollen tube to reach the embryo sac.

3.4 *Megasporogenesis and female gametophyte*

The female archesporium is single-celled. It functions directly as the megaspore mother cell and undergoes two meiotic divisions resulting in a linear tetrad of megaspores (figure 2A). The chalazal megaspore is functional while the micropylar three megaspores degenerate. The functional megaspore enlarges and its nucleus divides twice mitotically to give rise to 4 nuclei (figure 2 B,C). The embryo sac enlarges considerably and 4 nuclei divide to form 8-nucleate embryo sac of the Polygonum type. The micropylar nuclei organise into an egg apparatus and those at the chalazal end form the three antipodal cells. The remaining two polar nuclei fuse to form a secondary nucleus (figure 2D). The embryo sac is spindle-shaped. The synergids are hooked. The nucleus in the synergids is at the micropylar end while the distal part is occupied by a large vacuole. The antipodal cells are three in number and are uninucleate. They simulate egg apparatus in arrangement.

3.5 *Fertilisation*

Fertilisation is porogamous. The synergids degenerate soon after fertilisation (figure 2E).



Figures 2A–E. **A.** Megaspore tetrad; **B, C.** Two- and four-nucleate embryo sacs. **D.** LS mature embryo sac; **E.** Upper part of embryo sac, degenerating synergids, zygote and primary endosperm nucleus.

4. Discussion

The results obtained here agree with those obtained from earlier studies of the embryology of other representatives of the family. However, the statements of Snow (1945) and Anderson (1970) on *Chrysothamnus*, Tiagi and Taimni (1963) on *Vernonia*

cinerea and *V. cinerascens*, Walter and Kuta (1971) on *Sonchus oleraceus* and *S. asper*, Kaul (1972, 1973) on *Hypochoeris radicata* and *Youngia japonica* that the anther tapetum is of the Glandular type appears questionable, since *Cicerbita alpina* studied here and other members studied by earlier authors (see Pullaiah 1984) show a periplasmodial tapetum.

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Dissimilar chromosome pairing pattern in related populations of tetraploid pearl millet

P S R L NARASINGA RAO and K ARUNDHATI
Department of Botany, Andhra University, Waltair 530 003, India

MS received 11 July 1983

Abstract. Established populations of crop plant autotetraploids all possess multivalents. However, there is a seeming excess of bivalents in many instances, which is not readily accounted for in theory. To the small number of species, among the auto-tetraploids of which there is quantified information concerning chromosome pairing pattern may now be added *Pennisetum typhoides* (Burm.) S. & H. (source diploid IP 1475).

Keywords. Bivalent prevalence; autotetraploids; *Pennisetum typhoides*; chromosome pairing

1. Introduction

Though trivalents and univalents are scarce in established/viable autotetraploids, quadrivalents are unavoidable. Chromosome structural repatterning, as a factor in promoting bivalent type of pairing at the expense of multivalents in autotetraploids is a subject much studied and Sybenga (1973) concluded that as a practical means to preclude multivalency (allopolyploidization of autopolyploids) the method has no promise. Another long recognized factor limiting to multivalency (even in fully homologous quartets of chromosomes) is chiasma localization (Levan 1940; Shaver 1962; Sybenga 1975). When each chromosome arm is about one pairing block, and each forms a chiasma, any multivalents formed are simple unbranched configurations. Furthermore, the probability of partner exchange in a given quartet of (nearly) metacentric chromosomes, with one terminal chiasma per arm and no dearth of chiasmata per cell, is 0·67 such that the eventual multivalent number equals the bivalent number (John and Henderson 1962; Sved 1966; Venkateswarlu 1950). However, multivalency for two-thirds of a tetraploid's chromosomes (Morrison and Rajathy 1960) is not a general rule (Roseweir and Rees 1962; Timmis and Rees 1971). Fewer multivalents are observed in natural and induced tetraploids of *Dactylis* (Mc Collum 1958) or are achieved with generation advance in tetraploid maize (Gilles and Randolph 1951). Furthermore, even in autotetraploid populations of rye characterized by positive correlation between "seedset-chiasmata-quadrivalents", Timmis and Rees (1971) reported a "bivalent excess". Corresponding patterns to the last mentioned case are visualized in tetraploids of *Lolium*, *Festuca* (Simonsen 1973, 1975) *Adiantum* (Verma 1977) and now in tetraploids of pearl millet, *Pennisetum typhoids* (Burm.) S. & H.

2. Materials (their breeding history)

The present materials are from a selection experiment which is described elsewhere (Arundhati *et al* 1983) and only briefly restated here. Four C_5 generation populations of

tetraploids, P_1 to P_4, gave rise to twelve derived C_7 samples P_1H, P_1L and P_1US to P_4, P_4L and P_4US (H = high fertile, L = low fertile and US = unselected). Data from acetocarmine stained pollen mother cells (pmc) at diakinesis were from 20 cells each from each of the 8 plants in derived populations. All plants were autotetraploids with $4n = 28$ chromosomes. At diakinesis the smallest and the nucleolar quartet is readily recognized, and is left out of analysis where the interest was mainly in the nearly metacentric chromosomes of the first six quartets (all these are known to form branched trivalents as seen in the primary trisomic studies of Virmani and Gill 1971).

In the cited selection experiment there were three consistent features: (i) decrease of tri- and univalents, (ii) increase of the proportion of anaphase I pmcs with 14-14 distribution of chromosomes, and (iii) increase of quadrivalents at the expense of lower associations in such populations where there was increased chiasma frequency. In the following account two questions are examined: (i) the relation of quadrivalents and bivalents to chiasmata, and (ii) the tendency against partner exchanges in some families.

3. Results and discussion

The relation of quadrivalents IV, bivalents II, and trivalents III on the one hand and chiasmata on the other was studied by computing the regressions in two ways: (i) based on mean values from each of the 8 plants within each family, and (ii) based on pooling all pmcs of each family which are then reclassified on the basis of chiasma number per cell. The two are called $b1$ = regression-plant means and $b2$ = regression-cell population means (table 1). In only a few families are there significant $b1$ regressions, in each case showing a positive relation between IV and chiasmata. That such a IV-chiasmata relation is basic to these tetraploids is brought out from $b2$ regressions. The IV-chiasmata regressions based on cell population means $b2$ were significantly positive in all the samples. This is reflected in the III-chiasmata relationship; $b1$ regressions were not significant in general but $b2$ regressions were significantly negative in many samples (quadrivalent gain is at the expense of lower associations). Noteworthy is the lack of positive association between bivalents and chiasmata at the plant level in any family. Furthermore, at the cell level, the $b2$ II-chiasmata relationship is significantly negative in some families ($p < 0{\cdot}05$ in one case and $p < 0{\cdot}01$ or $0{\cdot}001$ in most). The exceptions are essentially in the P_1 and P_4 groups. In P_1 (C_5) the $b2$ for II-chiasmata was negative ($p < 0{\cdot}001$) but derived P_1 samples (C_7) show no relationship. In P_4 lack of $b2$ relationships is true for source (C_5) as well as derived samples (C_7).

This suggests that the plant genotype taken as a whole is limiting to the expression of the IV-chiasmata correlation (Mc Collum 1958; Hossain and Moore 1975). Cell level analysis jumbles the plant genotypes and pooled cells present a more straightforward picture of the correlation, freed as it were from the influence of individual genotypes (now nullifying each other due to scrambling). Thus chiasma increase implies frequent partner exchanges but this feature is hampered by the plant genotype as a whole (comparison of $b1$ and $b2$ regressions).

In the present plants the average chiasma frequency per bivalent is in general about 1·65, whereas the average chiasma frequency per quadrivalent is consistently more than double that value, being 3·8 or more (figures 1–6). In view of this trend it was noted (Narasinga Rao and Pantulu 1982) that tetraploid pearl millet is not well suited for analysis based on the John and Henderson model, because one condition is not

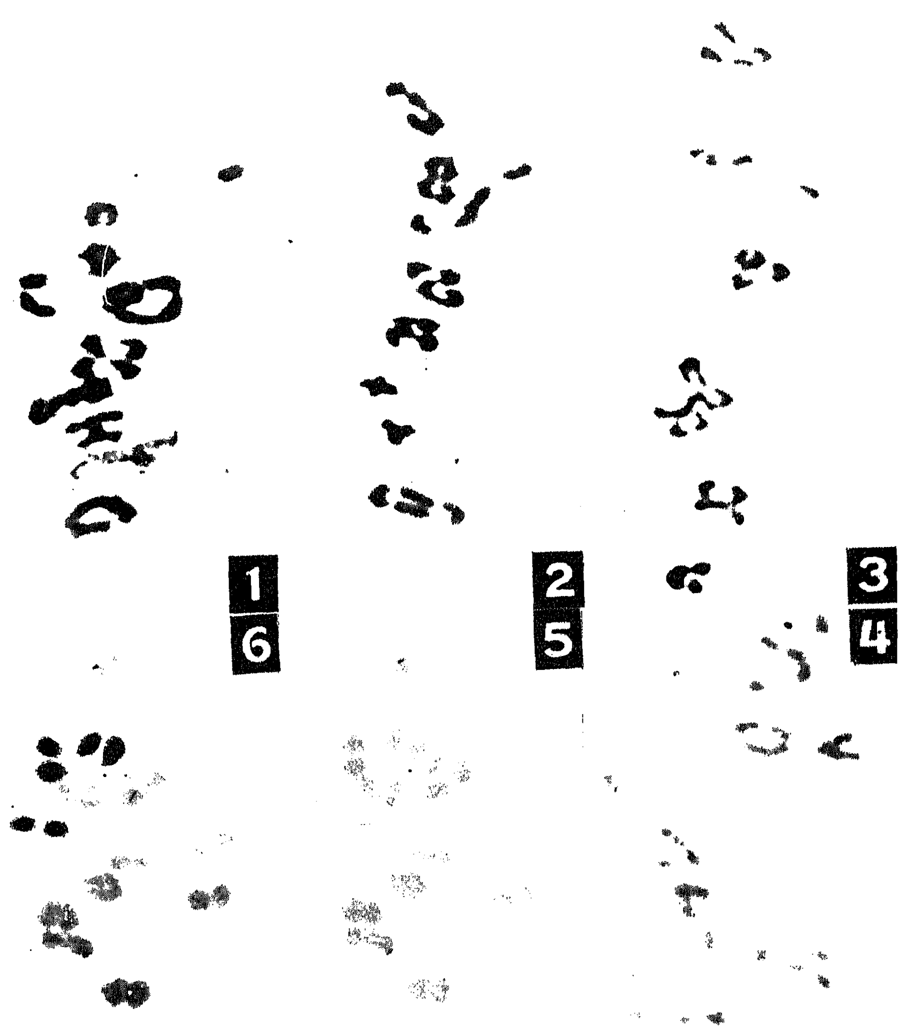
Table 1. Regression coefficients, $b1$ and $b2$, depicting the relation between quadrivalents, bivalents and trivalents on one hand and chiasmata on the other in 16 samples of autotetraploids†.

Sample name	Regression coefficients: Quadrivalents and chiasmata		Bivalents and chiasmata		Trivalents and chiasmata	
	$b1$	$b2$	$b1$	$b2$	$b1$	$b2$
C_5 P_1	$+0{\cdot}41^{a}$	$+0{\cdot}32^{c}$	$-0{\cdot}68^{ns}$	$-0{\cdot}42^{c}$	$-0{\cdot}06^{ns}$	$-6{\cdot}83^{a}$
P_2	$+0{\cdot}34^{b}$	$+0{\cdot}27^{c}$	$-0{\cdot}36^{ns}$	$-0{\cdot}25^{b}$	$-0{\cdot}12^{c}$	$-0{\cdot}14^{c}$
P_3	$+0{\cdot}32^{a}$	$+7{\cdot}59^{a}$	$-0{\cdot}27^{ns}$	$-0{\cdot}45^{b}$	$-0{\cdot}09^{ns}$	$-0{\cdot}23^{a}$
P_4	$+0{\cdot}19^{ns}$	$+0{\cdot}23^{c}$	$-0{\cdot}26^{ns}$	$-0{\cdot}17^{ns}$	$+0{\cdot}08^{ns}$	$-0{\cdot}12^{b}$
C_7P_1H	$+0{\cdot}15^{ns}$	$+0{\cdot}14^{b}$	$-0{\cdot}18^{ns}$	$-0{\cdot}16^{ns}$	$+0{\cdot}09^{a}$	$-6{\cdot}54^{a}$
P_1L	$+0{\cdot}09^{ns}$	$+0{\cdot}25^{c}$	$-0{\cdot}83^{ns}$	$-0{\cdot}28^{b}$	$-0{\cdot}04^{ns}$	$-4{\cdot}72^{ns}$
P_1US	$+0{\cdot}02^{ns}$	$+0{\cdot}23^{c}$	$-0{\cdot}48^{ns}$	$-0{\cdot}28^{ns}$	$+0{\cdot}04^{ns}$	$-7{\cdot}85^{ns}$
P_2H	$+0{\cdot}27^{ns}$	$+0{\cdot}29^{c}$	$-0{\cdot}31^{ns}$	$-0{\cdot}23^{b}$	$-0{\cdot}04^{ns}$	$-0{\cdot}10^{a}$
P_2L	$+0{\cdot}15^{ns}$	$+0{\cdot}28^{b}$	$-0{\cdot}15^{ns}$	$-0{\cdot}31$	$-0{\cdot}03^{ns}$	$-6{\cdot}24^{ns}$
P_2US	$+0{\cdot}05^{ns}$	$+0{\cdot}20^{b}$	$+0{\cdot}26^{ns}$	$-0{\cdot}26^{ns}$	$-0{\cdot}13^{b}$	$-9{\cdot}50^{b}$
P_3H	$+0{\cdot}39^{a}$	$+0{\cdot}23^{c}$	$-0{\cdot}76^{a}$	$-0{\cdot}36^{c}$	$-0{\cdot}01^{ns}$	$-5{\cdot}09^{ns}$
P_3L	$-0{\cdot}10^{ns}$	$+0{\cdot}14^{b}$	$+0{\cdot}38^{ns}$	$+0{\cdot}06^{ns}$	$-0{\cdot}07^{ns}$	$-0{\cdot}14^{c}$
P_3US	$+0{\cdot}29^{ns}$	$+0{\cdot}25^{c}$	$-0{\cdot}17^{ns}$	$-0{\cdot}34^{b}$	$-0{\cdot}15^{b}$	$-5{\cdot}18^{ns}$
P_4H	$+0{\cdot}26^{ns}$	$+0{\cdot}19^{c}$	$-0{\cdot}47^{ns}$	$-0{\cdot}14^{ns}$	$-0{\cdot}02^{ns}$	$-0{\cdot}12^{ns}$
P_4L	$+0{\cdot}03^{ns}$	$+0{\cdot}18^{b}$	$+0{\cdot}03^{ns}$	$-0{\cdot}04^{ns}$	$-0{\cdot}07^{ns}$	$-0{\cdot}11^{b}$
P_4US	$+0{\cdot}27^{ns}$	$+0{\cdot}22^{c}$	$-0{\cdot}30^{ns}$	$-0{\cdot}16^{ns}$	$-0{\cdot}04^{ns}$	$-0{\cdot}02^{ns}$

$b1$ = based on plant means and $b2$ = based on cell population means
† belonging to 4 categories, 3 of which ($P_1 P_2$ and P_4) had a common ancestor. ^{a} $p < 0{\cdot}05$; ^{b} $p < 0{\cdot}01$; ^{c} $p < 0{\cdot}001$; ns–not significant

satisfied, that there be an optimum of one chiasma per paired arms (note: branched multivalents). Nevertheless, the model was employed (table 2), along the lines used by Timmis and Rees (1971): cells with a dearth of chiasmata are avoided by selecting only those with not more than one rod bivalent and those where any univalents present are assignable to accompanying trivalents Sybenga (1975), commended caution concerning this approach: in species where "quadrivalents . . . have a lower chiasma frequency than bivalents, the selected group automatically excludes cells with high frequencies of (potential) quadrivalents". Among the present plants average frequencies of chiasmata in bivalents and quadrivalents in P_1 and P_4 families are as follows: II 1·54 and IV 3·89 in P_1; II 1·55 and IV 3·77 in P_4; II 1·63 and IV 3·97 in P_1H and II 1·64 and IV 3·80 in P_4H samples. (similar were the values in P_2 and P_3 groups).

As an expected corollary of plant level prevalence of quadrivalents, even the selected cells revealed quadrivalent excess in many families (table 2). However, the few families where bivalent increase was observed in C_7 generation were indeed the ones in whose selected cells there was a shortage of quadrivalents and an excess of bivalents against the 1:1 expectation (it is recalled that the predisposition to branched multivalent formation in this species renders the 1:1 expectation inaccurate: more than 1 IV *vs* less than 1 II is the more realistic expectation). The superficial appearance that the relative low chiasma frequencies in P_4 group are responsible for fewer multivalents is thus falsified. In cell



Figures 1–6. Pollen mother cells (at first metaphase) from autotetraploids ($4n = 28$), displaying a progressive decrease of branched quadrivalents and unterminalized chiasmata. **1.** 5 quadrivalents, 1 trivalent + univalent and 2 ring bivalents. **2.** 6 quadrivalents and 2 rod bivalents. **3.** 7 quadrivalents. **4.** 6 quadrivalents and 1 trivalent + univalent. **5.** 14 bivalents (11 rings and 3 rods). **6.** Explanatory diagram of figure 5; heavily marked in black ink are the three rod bivalents and the rest are ring bivalents.

samples without a dearth of chiasmata there exists a trend against partner exchanges. Divergence in this respect is indicated from the fact that P_1, P_2 and P_4 groups had a common parent, C_0, the induced tetraploid (diploid source 1P 1475).

It would seem that one comparable aspect between bivalent forming allopolyploids (Sears and Okamoto 1958; Riley and Chapman 1958) and well established (fairly fertile) autotetraploids is the control/restriction on partner exchanges; ruled out (in VB presence) in the allopolyploid's chromosomes and much restricted (distal localization)

Table 2. Results of χ^2 tests comparing observed frequencies of quadrivalents IV and bivalents II per cell in selected cell samples against the John and Henderson model.

Sample Name	No. of selected cells†	Observed and expected quadrivalents IV and bivalents II⁺			χ^2	
		IV	II	Expectation†	IV	II
P_1	43	191	134	172	$2{\cdot}10^{ns}$	$8{\cdot}40^{b}$
P_2	18	74	68	72	$0{\cdot}06^{ns}$	$0{\cdot}22^{ns}$
P_3	30	118	124	120	$0{\cdot}03^{ns}$	$0{\cdot}13^{ns}$
P_4	21	88	76	84	$0{\cdot}19^{ns}$	$0{\cdot}76^{ns}$
P_1H	131	614	344	534	$11{\cdot}99^{c}$	$67{\cdot}60^{c}$
P_1L	30	126	108	120	$0{\cdot}30^{ns}$	$1{\cdot}20^{ns}$
P_1US	61	247	238	244	$0{\cdot}03^{ns}$	$0{\cdot}15^{ns}$
P_2H	44	155	218	176	$2{\cdot}51^{ns}$	$10{\cdot}02^{b}$
P_2L	24	81	126	96	$2{\cdot}34^{ns}$	$9{\cdot}38^{b}$
P_2US	14	51	66	56	$0{\cdot}45^{ns}$	$1{\cdot}76^{ns}$
P_3H	96	377	398	374	$0{\cdot}02^{ns}$	$1{\cdot}53^{ns}$
P_3L	16	56	80	64	$1{\cdot}00^{ns}$	$4{\cdot}00^{a}$
P_3US	31	122	128	124	$0{\cdot}03^{ns}$	$0{\cdot}13^{ns}$
P_4H	32	103	178	128	$4{\cdot}88^{a}$	$19{\cdot}53^{c}$
P_4L	8	24	48	32	$2{\cdot}00^{ns}$	$8{\cdot}00^{b}$
P_4US	21	64	124	84	$4{\cdot}76^{a}$	$19{\cdot}05^{c}$

† Employing the Timmis and Rees (1971) treatment, as pertaining to the six longer chromosome quartets in pearl millet tetraploids. Expectation 4IV:4II. ⁺ Instances of bivalent excess underscored. ^{a} $p < 0{\cdot}05$; ^{b} $p < 0{\cdot}01$; ^{c} $p < 0{\cdot}001$; ns = not significant; D.F. = 1.

in the autotetraploids' chromosomes. Because the rye example worked out by Timmis and Rees (1971) is suggestive of a mechanism tending to preclude partner exchanges, be it in respect of only a part of the chromosome complement, such seeming specializations are worth screening for among the various genotypes of crop plant tetraploids. Further development of models and analysis (Jackson and Casey 1982) might shed some more light on this desirable feature of bivalent prevalence in autotetraploids.

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Floral biology of *Torilis leptophylla* (L.) Reichenb. f.

PUSHPA KOUL, A K KOUL and I A HAMAL
Department of Biosciences, University of Jammu, Jammu 180001, India

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Abstract. *Torilis leptophylla*, an andromonoecious umbellifer, practises a blend of geitonogamy and outbreeding. Although it exhibits strong dichogamy at the level of individual flowers, in the full plant, pistillate and staminate phases overlap. The ratio of hermaphrodite to staminate flowers is 0·37 which is intermediate to those recorded for other andromonoecious umbellifers.

Keywords. *Torilis leptophylla*; andromonoecious; geitonogamy.

1. Introduction

To understand patterns of genetic variation in natural populations, knowledge of breeding system biology is necessary. While some plant families exhibit uniformity in breeding system, others such as Umbelliferae (Apiaceae) include hermaphrodite, andromonoecious, gynodioecious and dioecious taxa which follow varied modes of reproduction. Braak and Kho (1958), Bell (1971), Kumar (1977), Lovett Doust (1980) and Webb and Lloyd (1980) have studied some umbellifers from this angle but the majority still await investigation. The present communication describes certain aspects of the breeding system of *Torilis leptophylla*, a common weed in Kashmir Himalayas from 1600 to 1900 m.

2. Material and methods

Floral biology and umbel constitution was determined from live plants from June to August 1982. The proportion of hermaphrodite and staminate flowers was determined for whole plants as well as at the level of umbel orders. The percentage of hermaphrodite and staminate flowers and the ratio between them have been worked out according to Lovett Doust (1980).

Fruits formed in umbels of different orders were counted and percentage fruit set calculated.

Floral biology, flower morphology and anthesis were studied at different time intervals. The number of pollen grains produced per anther was counted using aniline blue and lactophenol. Pollen-ovule ratios were then calculated for hermaphrodite flowers (which have 5 anthers and two ovules) and for individual plants when the pollen contribution of male flowers was also included in the estimation.

3. Results

Plants 20–70 cm tall, hispid, annual herbs; leaves two pinnate, segments linear-lanceolate; peduncles short, 2–4 cm in length, involucel of several linear bractlets. The

plants are andromonoecious with hermaphrodite and staminate flowers borne within the same umbel.

3.1 *Inflorescence*

The ultimate branches as well as the main stem terminate in an umbel which consists of 3–7 umbellets. Umbels up to third (tertiary) and rarely fourth (quarternary) order are produced. The percentage of umbels of different orders in an individual are: primary: 8·51, secondary: 31·91, tertiary: 46·81 and quarternary: 12·77.

Each umbellet bears hermaphrodite flowers at periphery and staminate flowers in the centre (figures 1, 2).

3.2 *Floral morphology*

The hermaphrodite flowers have a zygomorphic corolla. Of the five petals in a flower, one is large, two are small and other two are intermediate in size. The five stamens alternate with the petals. Anthers are dorsifixed. Pistil is bicarpellary and syncarpous. The ovary is inferior, bilocular and bears a pendulous ovule in each locule. The ovary is capped by a bulbous stylopodium from which emerge two styles (figures 3, 4).

Except for their smaller size and the absence of pistil and petal symmetry, the staminate flowers resemble their hermaphrodite counterparts in all respects (figures 5, 6).

3.3 *Anthesis*

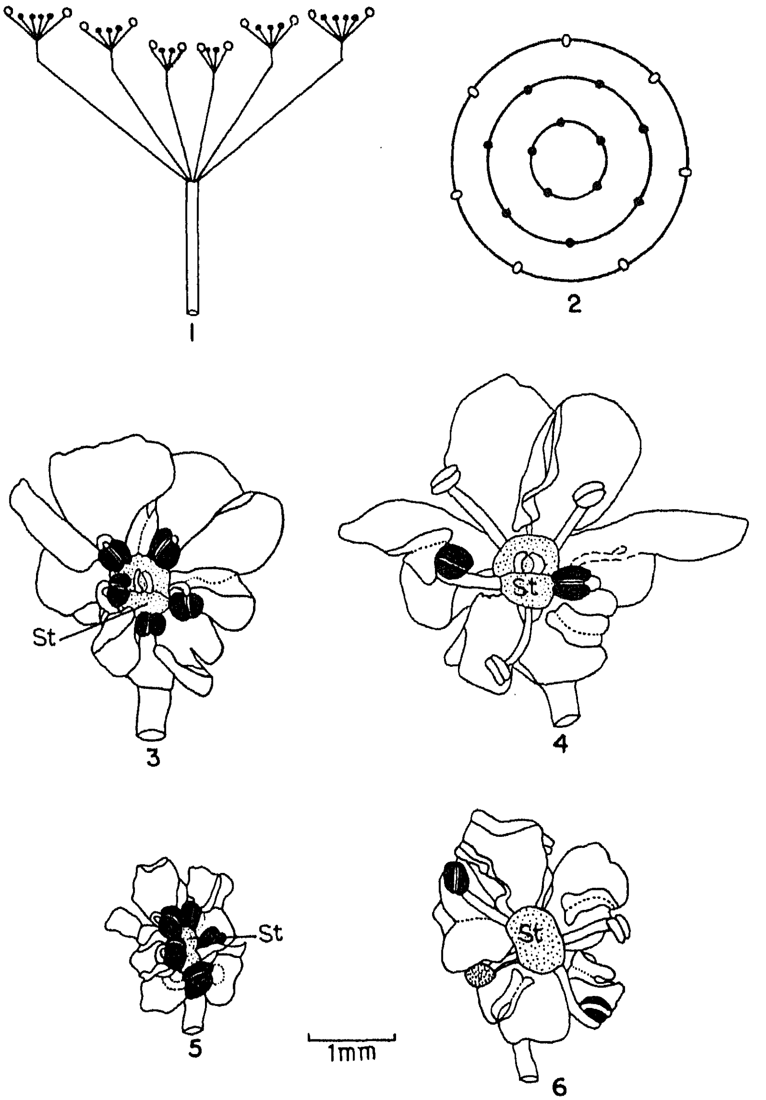
The inflorescence differentiates within the leaf sheath. As it emerges, the umbellets fan out in sequence. Flowers of the primary umbel are first to bloom, followed by those of the secondary, tertiary and quarternary umbels. In each umbellet, the hermaphrodite flowers mature earlier than the staminate flowers. An umbel takes 4–6 days (mean $4·8 \pm 0·84$) to bloom. In hermaphrodite flowers, the stamens mature earlier than the pistil so that the species is protandrous. The stigma becomes receptive much later, when all anthers have dehisced. The anther dehisces through two longitudinal slits. When flowers in second order umbel start opening, the stigmas of the flowers of first order umbel are still receptive. The same sequence is repeated in subsequent umbel orders as well.

3.4 *Proportion of hermaphrodite and staminate flowers*

In a mature plant, the number of staminate flowers exceeds that of hermaphrodite flowers (table 1); the average ratio is 0·37 (1 hermaphrodite: 2·73 staminate). The proportion of staminate and hermaphrodite flowers varies in umbels of different orders and this variation is reflected in fruit set as well (table 2). The percentage fruit set is maximum in primary umbel and it progressively decreases in umbels of lower orders.

3.5 *Pollen-ovule ratio*

In andromonoecious species, pollen grains are formed in both flower types, but ovules are produced by hermaphrodite flowers only. The mean total number of pollen grains produced per hermaphrodite flower is 5,301 ($\pm 458·3$) against a total of 2 ovules. The



Figures 1–6. **1–2.** Diagrammatic representation of the arrangement of hermaphrodite flowers (blank circles) and staminate flowers (solid circles) in (1) umbel, and (2) umbellet; **3.** Hermaphrodite flower bud with incurved stamens; **4.** Hermaphrodite flower with its parts exposed; **5.** Staminate flower bud; **6.** Staminate flower showing reduced stylopodium and relatively smaller petals (St = Stylopodium).

pollen-ovule ratio for a hermaphrodite flower is 2650·5; but, for whole plant, the ratio averages to 9883.

4. Discussion

Like other andromonoecious umbellifers, *Torilis leptophylla* too exhibits variation in the proportion of hermaphrodite and staminate flowers in umbels of different orders.

Table 1. Mean values for staminate and hermaphrodite flowers and fruit set in a plant.*

Character	Frequency
Total no. of flowers	1197·10 ±233·71 (744 – 1471)
No. of hermaphrodite flowers	320·80 ±60·11 (239 – 407)
Percentage of hermaphrodite flowers	26·80 ±3·58 (24·64 – 36·56)
No. of staminate flowers	876·30 ±182·95 (472 – 1065)
Percentage of staminate flowers	73·20 ±3·58 (63·44 – 75·33)
Ratio of ⚥ : ♂ flowers	0.37
Total no. of fruits formed	295·30 ±52·67 (224 – 368)
Percent fruit set of ⚥ flowers	91·54 ±2·45 (88·24 – 96·98)

* Mean of 20 determinations; values in parentheses represent ranges.

Table 2. Quantitative data (mean ±SD) on staminate and hermaphrodite flowers and fruit set in umbels of different orders.

Character	Umbel order			
	Primary	Secondary	Tertiary	Quarternary
No. of umbels studied	20	75	110	12
Total no. of flowers	113·70 ±9·64 (94 – 124)	484·80 ±98·50 (302 – 559)	581·80 ±147·25 (348 – 815)	43·00 ±15·85 (29 – 60)
No. of hermaphrodite flowers	49·20 ±7·98 (34 – 59)	138·10 ±24·03 (100 – 166)	129·00 ±38·73 (91 – 195)	11·25 ±6·85 (5 – 19)
Percentage of hermaphrodite flowers	43·27 ±4·90 (36·17 – 49·58)	28·51 ±4·44 (25·00 – 40·73)	22·17 ±4·35 (18·58 – 33·03)	26·16 ±6·80 (17·24 – 31·62)
No. of staminate flowers	64·50 ±6·40 (60 – 76)	346·30 ±79·67 (179 – 408)	452·80 ±115·08 (233 – 620)	31·75 ±9·03 (24 – 41)
Percentage of staminate flowers	56·73 ±4·90 (50·42 – 63·83)	71·49 ±4·44 (59·27 – 75·00)	77·83 ±4·35 (66·95 – 81·42)	73·84 ±6·80 (68·33 – 82·76)
Ratio of ⚥ ♂ flowers	0·87	0·40	0·28	0·35
Total no. of fruits formed	49·20 ±7·98 (34 – 59)	134·00 ±7·44 (97 – 160)	110·70 ±9·19 (83 – 160)	3·50 ±4·12 (0 – 8)
Percent fruit set of ⚥ flowers	100	97·03 ±0·43 (93·50 – 98·53)	85·81 ±1·72 (79·13 – 94·74)	27·80 ±25·15 (0·0 – 42·10)

Values in parentheses represent the ranges.

In some taxa such as *Daucus carota* there is a gradual decrease in frequency of hermaphrodite flowers in lower order umbels (Bell 1971). Others like *Scandix pecten-veneris* show a decrease in second order umbels followed by an increase in umbels of lower orders, the last formed umbel again showing decrease (author's unpublished data). In *Anthriscus sylvestris*, *Pastinaca sativa* and *Smyrnium olusatrum*, there is gradual but continued decrease with the last formed umbels producing only male flowers (Lovett Doust 1980). In *T. leptophylla*, the highest percentage of hermaphrodite flowers exists in primary, followed by a gradual decrease in lower order umbels. In each umbel order, the male or polliniferous flowers outnumber their bisexual counterparts.

The proportion of staminate and hermaphrodite flowers in an umbel bears a relationship to pollen loss caused by insect visits to an inflorescence. Large-sized umbels of *A. sylvestris* and *P. sativa* possess a much higher percentage of staminate flowers in their inflorescences (1 hermaphrodite: 4 staminate). These inflorescences attract insects in large numbers. On the contrary, small and generally simple umbels of *S. pecten-veneris* have a significantly low frequency of polliniferous flowers (1 hermaphrodite: 1·07–1·2 staminate). On account of their poor visual impact, these umbels are not frequented by insects. Umbels of *T. leptophylla* are medium sized which gets reflected even in the proportion of staminate and hermaphrodite flowers per umbel (1 hermaphrodite: 2·73 staminate). Plants of this species suffer some amount of pollen loss due to insect visitors but this loss is compensated by the nearly three-fold frequency of staminate flowers.

A higher number of male flowers in the umbel boosts pollen production and promotes insect visits during which pollination is effected. It also ensures the availability of male sex cells for fertilization. Paternal fitness is limited by access of male sex cells to female (Bateman 1948). Staggering in the maturation time of pollen increases the period for which the pollen becomes available for fertilization (Lloyd 1979).

Another significant feature in plant breeding systems is the proportion in which pollen grains and ovules are produced by an individual. Low pollen-ovule ratios characterise autogamous species (Cruden 1977). In *Torilis leptophylla*, the pollen-ovule ratio per hermaphrodite flower is around 2650, but at the level of the whole plant the ratio works out to 9883 which conforms to the figures compiled for xenogamous or outbreeding taxa by Cruden (1977).

Such structural features of *Torilis* flower as the zygomorphic corolla (in hermaphrodite flowers), exposed nectar, prominent stylopodium and co-existence of perfect and staminate flowers are suggestive of its outbreeding nature. Moreover, the plants show dichogamy which is also a contravance for outbreeding. However, since staminate and pistillate phases overlap when the plant is considered in totality, obligate outcrossing is ruled out. Although umbellifers are in general self-compatible (Bell 1971), selfing is not possible within individual flowers of *Torilis leptophylla* because they are protandrous. Evidences such as umbel constitution, structural adaptation of flower, pollen-ovule ratio and relative maturation of male and female sex-cells suggest that *Torilis leptophylla* experiences both geitonogamy as well as out-crossing.

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Antirrhinum orontium complex: biosystematic studies

CHARANJIT MAHAL and M PAL
National Botanical Research Institute, Lucknow 226 001, India

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Abstract. *Antirrhinum orontium*, a widely distributed species exists in a number of forms, four of which are studied here. The three European forms are related morphologically and are inter-fertile but the local taxon is not only morphologically different from its European counterparts, but also genetically distinct. It is suggested that the taxon merits a specific status.

Keywords. *Antirrhinum orontium*; biosystematics.

1. Introduction

A. orontium Linn, originally a native of southern Europe, is widely distributed all over Europe, north Africa and Central Asia up to the Western Himalayas (Hooker 1885; Koch 1907). In the northwest Indian plains one of its varieties is a common winter weed (Hooker 1885; present observations). Commensurate with its wide geographic distribution the species is morphologically heterogeneous. The Indian variety of this species is studied morphologically and cytogenetically and compared with its European counterparts. While the authorities at the Royal Botanic Gardens, Kew, identified the Indian variety as *A. orontium* Linn var *indicum* Chav, the European material received as varieties *parviflorum* DC F and *grandiflorum* Chav from Hortus Botanicus Coimbra, Portugal and the Botanic Garden, Cambridge, England respectively were both identified as *A. orontium* L var *orontium*. However due to morphological differences and also for convenience, these have been designated by their original names in this study. Another species *A. calycinum* Lam obtained from Hortus Botanicus, Coimbra, Portugal, is morphologically very similar to *A. orontium* var *grandiflorum*.

2. Observations

2.1 *Morphology*

A perusal of the morphological features of the three varieties, (table 1) reveals that *A. orontium* var *indicum* Chav differs from the other two in being dwarf, less branched, with fewer leaves which are smaller and narrower, white corolla and a thinner capsule (figures 1–4).

2.2 *Meiosis and crossability*

Analysis of a large number of pollen mother cells of *A. calycinum* and of the three varieties of *A. orontium* shows that meiosis is perfectly normal in all the taxa. There are

Table 1. Summary of chief morphological characters of *Antirrhinum orontium* complex.

Taxon	Habit	Height (cm)	Leaf size $l \times b$ (cm)	Corolla Colour	Corolla Size $l \times b$ (cm)	Capsule $l \times b$ (cm)
var *indicum* chav.	Sparsely branched, 2–4 branches arising on main shoot near the base; very hairy on all parts except leaves. Leaves few.	40–60	5·4 × 0·2	White	1 × 0·55	Long with a rounded base 0·8 × 0·4
var *parviflorum* chav	Bushy with a number of branches. All very leafy. Hairy all over except leaves.	102–120	6·7 × 1·2	Violet with deeper violet streaks on upper lip	1·5 × 1·0	Almost globose 0·9 × 0·8
var *grandiflorum* chav (including *A. calycinum* Lam)	Like parviflorum but completely devoid of hair.	115–130	5·5 × 0·9	White with violet streaks on upper lip	1·8 × 1·4	Almost globose 0·75 × 0·7

eight bivalents at diakinesis and metaphase I (figures 5–8) with normal (8:8) disjunction at both the anaphases leading to regular tetrad formation and a high percentage of stainable pollen.

The mean chiasma frequency per cell was minimum in *A. calycinum* (table 2: $8{\cdot}52 \pm 0{\cdot}07$) and maximum in *A. orontium* var *parviflorum* ($13{\cdot}2 \pm 0{\cdot}18$).

The taxa were crossed in all directions, while crosses between *A. calycinum* and *A. orontium* var *parviflorum* and var *grandiflorum* succeeded and gave rise to fertile, healthy F_1 and F_2, those with *A. orontium* var *indicum* failed in all directions. When a large number of pollinations did not succeed, pollen tube studies were undertaken on varieties of *indicum* and *parviflorum* after reciprocal pollination. Pollen grains of one variety readily germinate on the stigma of the other and pollen tubes grow unhindered down the style up to the ovary. The results were similar in the reciprocal crosses of the autotetraploids of the two species. Further studies were however not made, therefore it is not possible to state precisely at what stage the development is restricted—before or after fertilization.

3. Conclusions

The species *A. orontium*, with its small unattractive flowers is self-compatible, differing in this respect from most of the other species of the genus (Mahal 1972). Late in the season, the flowers become smaller in size and also practically cleistogamous.

According to Saunders (1927), *A. orontium* appears under only one form, having pale red flowers and hairy stems and capsules. On morphological grounds she considers *A. calycinum* as distinct from *A. orontium*, in having flowers that are intermediate in size between *A. orontium* and *A. majus*. Based on the colour of the corolla and hairiness she further distinguishes five forms under *A. calycinum*. However

Figures 1–8. 1. *A. orontium* var *indicum*. **2.** *A. orontium* var. *parviflorum*. **3.** Calyse, capsules and leaves of (top to bottom) var *indicum* and *parviflorum* and leaf of var *grandiflorum*. **4.** Flowers of (top to bottom) vars *indicum*, *parviflorum* and *grandiflorum*. **5–8.** MI 8 II. **5.** (var *indicum*). **6.** var *parviflorum*. **7.** var *grandiflorum*. **8.** *A. calycinum* (× 1800).

in the present study it was noted that *A. orontium* var *parviflorum* and *A. orontium* var *grandiflorum* are morphologically very similar, differing only in their flower colour and size and hairiness character. Whereas var *parviflorum* is very hairy, the latter variety is completely glabrous. *A. calycinum* is identical to var *grandiflorum*. Except for the reduced number of chiasmata (table 2) in *A. calycinum* no other difference was observed. Interpollinations carried out among the two varieties and *A. calycinum* showed that they cross readily and segregate in F_2 into forms similar to those described by Saunders. It would therefore be proper to maintain the two varieties *parviflorum* and *grandiflorum* separated by minor differences and *A. calycinum* as a synonym of var

Table 2. Summary of meiosis and pollen fertility in *A. orontium* complex.

	Taxon			
	A. orontium var *indicum*	var *parviflorum*	var *grandiflorum*	*A. calycinum*
Chromosome number (n)	8	8	8	8
No. of PMC	25	25	50	50
Xta				
Mean/cell	$12{\cdot}2 \pm 0{\cdot}23$	$13{\cdot}2 \pm 0{\cdot}18$	$10 \pm 0{\cdot}19$	$8{\cdot}52 \pm 0{\cdot}07$
Mean/bivalent	1·53	1·65	1·26	1·06
Pollen fertility (%)	97	87·98	90·3	90·3

grandiflorum. The local taxon identified as *A. orontium* var *indicum*, however, is clearly distinct from the first two, being significantly smaller and with fewer number of branches. It also differs in the shape and size of leaves and the smaller size of floral parts. In its crossability behaviour too, it differs from the other varieties. While var *parviflorum* and var *grandiflorum* readily cross with one another in both directions var *indicum* does not cross with either variety even after extensive pollinations, both when used as a seed parent or pollen parent. Crosses failed even at the tetraploid level between varieties *indicum* and *parviflorum*.

Thus *A. orontium* var *indicum* is reproductively isolated from the other two varieties of the species described here and is thus genetically distinct. Morphologically also it is distinct from the other varieties of *A. orontium*. The variety therefore is morphologically, geographically, and genetically distinct from the other varieties of the species and merits an elevation to a specific rank.

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Cosmarium botrytis Menegh under the light and scanning electron microscope

VIDYAVATI and G SATHAIAH

Department of Botany, Kakatiya University, Warangal 506 009, India

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Abstract. A detailed study of surface ornamentation under light and scanning electron microscope is undertaken. The present observations have added further to the knowledge in taxonomic identification of *Cosmarium botrytis* Menegh, in particular because the species revealed a variety of surface ornamentation based on which various species of *Cosmarium* are specifically identified.

Keywords. Taxonomy; *Cosmarium botrytis*; ornamentation; light microscope; scanning electron microscope.

1. Introduction

The genus *Cosmarium* occupies a very distinct place in the placoderm desmids (Conjugales, Chlorophyceae) of the green algae. As in other fields, SEM may prove of value in the taxonomy of the genus *Cosmarium* supplementing the data already obtained from LM (West and West 1908). SEM reveals clearly the plasticity of desmid morphology, which is of much importance to taxonomists.

SEM studies of desmids were first attempted by Lyon (1969) who studied the external morphology of the walls of *Cosmarium botrytis*, Pickett-Heaps (1973) presented stereo-scanning micrographs of two species of *Staurastrum*, one species of *Cosmarium* and two species of *Micrasterias*. Stereo-scanning studies indicate the potential value of this method for developmental studies and taxonomy. The cell wall of *Cosmarium botrytis* was studied (Lott *et al* 1972) using the conventional thin sectioning EM techniques and also SEM and freeze-etching. The freeze-etched preparations shattered the wall and showed overlapping of ribbons of microfibrils oriented randomly (Mix 1975). Recently, Vidyavati and coworkers (Vidyavati 1982a–e, 1983a–d; Vidyavati *et al* 1983) studied certain species of placoderm desmids under SEM. They also studied the ornamentations along with cell division in species of *Cosmarium*, *Euastrum* and *Staurastrum* under SEM. In this paper a detailed study of surface ornamentation in *Cosmarium botrytis* under the LM and SEM is undertaken.

2. Material and methods

Unialgal clonal cultures of *Cosmarium botrytis*, Menegh, (culture centre of Algae and Protozoa, Cambridge, UK) were raised in Chu's 10 (1946) inorganic medium subjected to a temperature of 18–22°C receiving 16/8 hr alternate light and dark periods, respectively.

Cells from the actively growing culture were embedded in a 10 % glycerine in a watch

glass. After one week, slides were prepared to study the front, lateral and top views of the species under the LM.

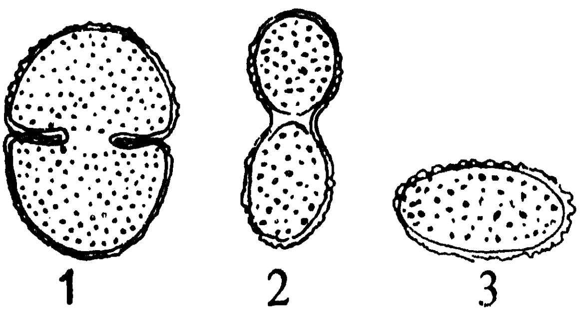
For SEM studies, actively growing cells were fixed in 1 % glutaraldehyde, made up in the culture medium, for about 1 hr at room temperature. After washing once in the culture medium, these cells were post-fixed for 1 hr in 2% OsO_4, also made up in culture medium. The cells were washed thrice with the culture medium. After fixation, the cells were dehydrated in various grades of acetone, (30, 50, 70, 90 and 100) and finally preserved in 100% acetone, kept in a desicator. The cells were dried by CPD (critical point drying) apparatus and mounted on a stub using double-sticking tape. The cells were coated with carbon and gold and observed through the SEM (Jeol JSM-25 S, at 15 kV).

Most of the desmids secrete copious quantities of mucilage and many of the species examined, were too thickly covered with dried material to give good micrographs. Washing fixed cells with warm water sometimes gave good results, but glusulase pre-treatment was more effective in removing the mucilage for SEM (Pickett-Heaps 1973, 1974; Vidyavati 1983d).

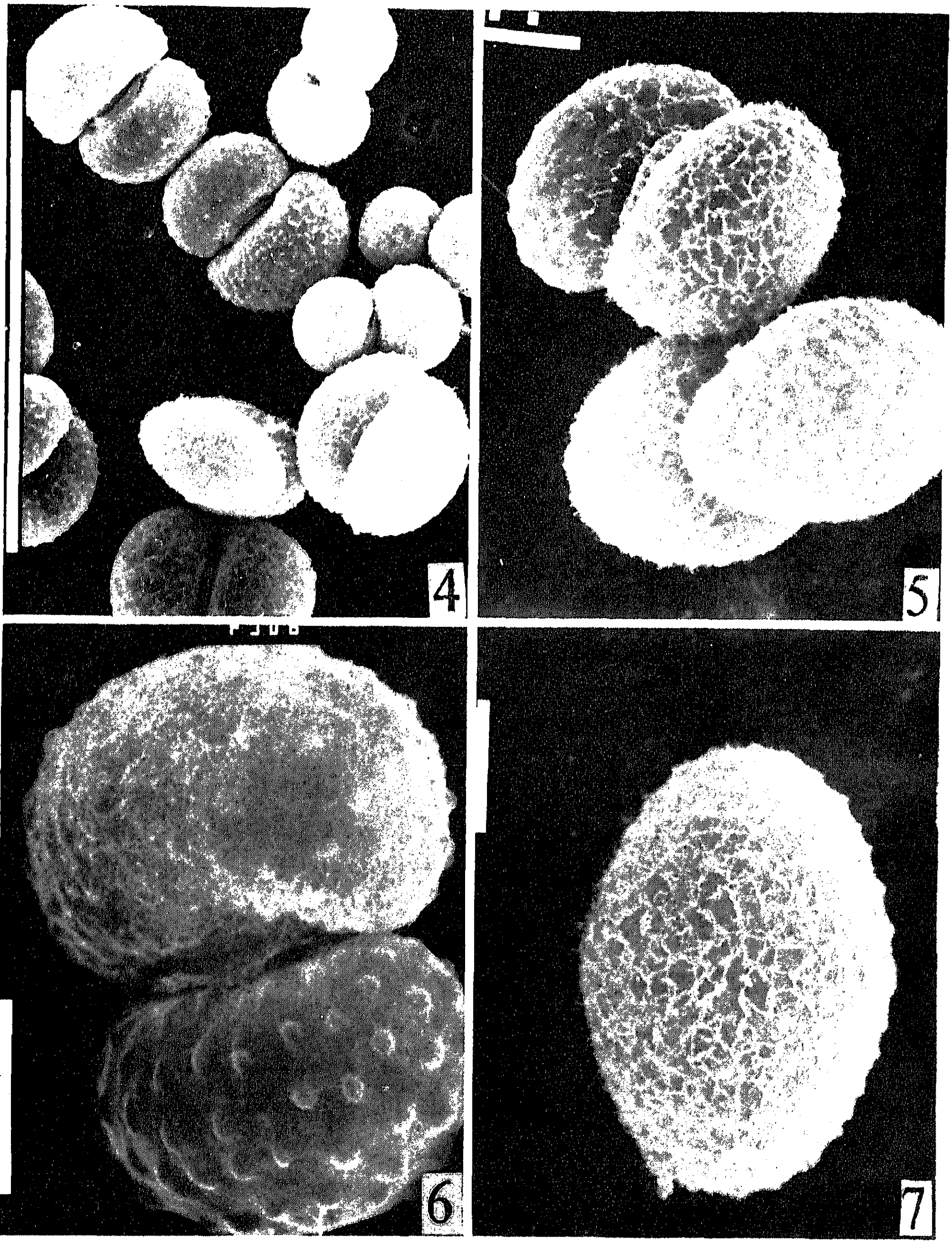
3. Observations and discussion

Figures (1–3) show the cells of various views (front, lateral and top) of *Cosmarium botrytis* under the LM. The (65–90 μm in length, 51–68 μm in breadth, 33–40 μm in thickness, breadth of isthmus 17–24 μm) cells were about $1\frac{1}{4}$–$1\frac{1}{3}$ times as long as broad, deeply constricted, sinus narrowly linear with a dilated extremity; semicells ovate pyramidate from a broad, flat base, basal (figures 1–3) angles rounded. Sides convex, apex rather narrowly truncate or subtruncate, apical angles rounded. Side view of semicell broadly elliptic (figure 2). Cell-wall uniformly granulate, granules somewhat small and generally without any definite deposition.

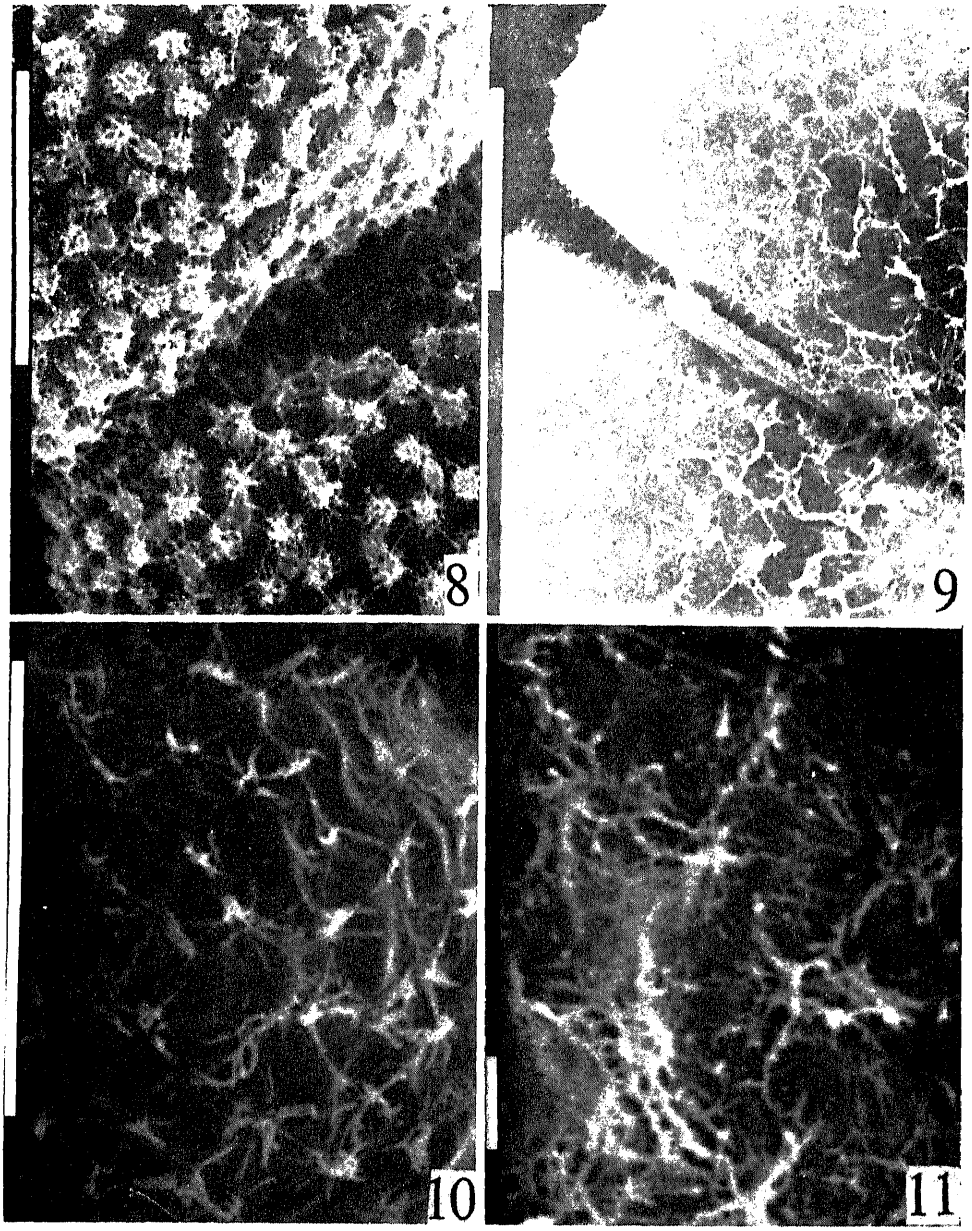
In *Cosmarium botrytis* the two semicells are covered by a variable pattern of warts or ridges and a net work of mucilage pores and strands. The secondary walls invariably possess a fairly regular pattern of mucilage deposits over the pores. Mucilage pores were connected by numerous mucilage strands. A number of small fibrils radiate from a mucilage pore (figures 14 and 15).



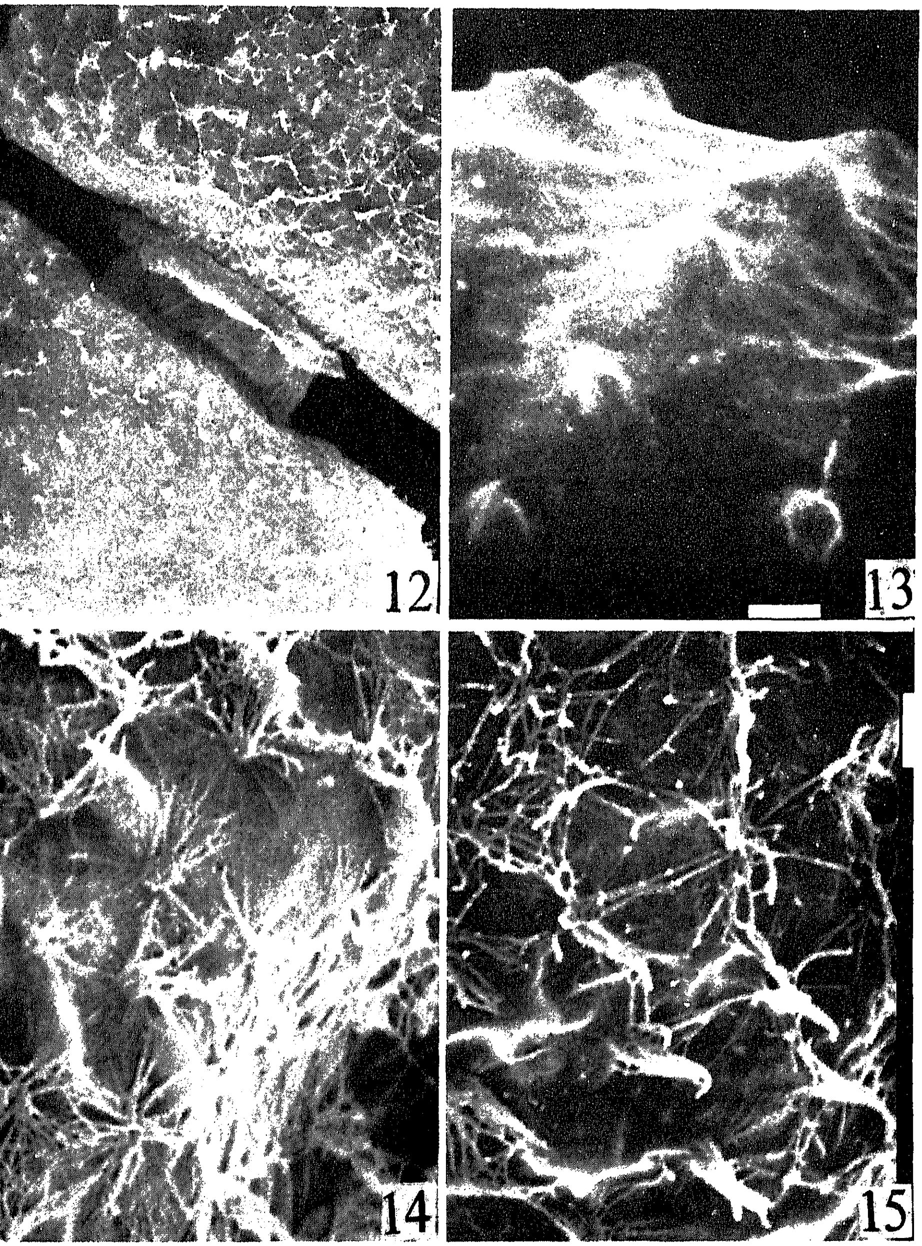
Figures 1–3. *Cosmarium botrytis* under LM (× 1200) showing the **1.** front view **2.** lateral view **3.** top view.



Figures 4–7. Scanning electron microphotographs of *Cosmarium botrytis* showing **4.** cells under various views at lower magnifications (front, lateral and top), **5.** cells in front and lateral views, **6.** a cell in side view, part of the semicell showing mucilage pores, **7.** the mucilagenous strands in the top view of the cell.



Figures 8–11. Scanning electron microphotographs of *Cosmarium botrytis*, showing the ornamentation at the isthmus region, **8.** mucilage accumulation at the isthmus, **9.** arrangement of mucilagenous strands after glusulase treatment, **10 and 11.** arrangement of mucilagenous strands at higher magnification.



Figures 12–15. Scanning electron microphotographs of *Cosmarium botrytis* showing **12.** enlargement of isthmus at the time of division, **13.** part of the cell wall showing mucilage pores and sheath collars, **14 and 15.** typical arrangement of mucilagenous strands.

Figure 4 shows cells under various views at low magnification, and figures 5–7 show the front, lateral and top views along with the cell wall beset with mucilage pores and strands. Whereas figures 8–11 show the cell with thick mucilage and mucilagenous pores and strands at the isthmus region. Figure 12 shows the enlargement of the isthmus during division and figure 13 a part of the inner secondary wall with mucilage pores and sheath collars. The pore is a simple canal with some slight enlargement internally, while externally it was a specially differentiated non-cellulosic cylindrical zone. It is known as 'pore organ' and at the inner surface of the pore entrance there are button-shaped swellings. Figures 14 and 15 show the regular pattern of mucilage deposits around the pores typical for the species. These observations further add to our previous knowledge (Pickett-Heaps 1975; Vidyavati *et al* 1983).

These studies have revealed the detailed surface ornamentation of *Cosmarium botrytis*, which showed strap-like arrays of parallel microfibrils (figures 14 and 15) like those mentioned in *Micrasterias* (Lott *et al* 1972; Mix 1975). However granules, spines or verrucae of varying size and elaboration are characteristic of the cell wall ornamentation of many desmids of the *Cosmariae*. They are arranged in regular and consistent patterns and can be clearly seen by SEM, which are significant in desmid taxonomy. However, they are not structures produced from the outer wall layer as is the ornamentation of the genera *Closterium*, *Penium* or *Gonatozygon*, but results from the differentiation of primary and secondary walls being initiated during the latter stages of cell enlargement in *Cosmarium botrytis*. Thus, they are intimately associated with the development of the cell wall proper. Finally all these observations have further added to the taxonomic identification of *Cosmarium botrytis*.

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Development and structure of ineffective nodules in some leguminous weeds

P S JAIN and PURNIMA SHRIVASTAVA
Department of Botany, University of Rajasthan, Jaipur 302 004, India

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Abstract. The structure and development of the ineffective nodules of *Tephrosia apollinea*, *Trigonella polycerata*, *Vicia hirsuta* and *V. sativa* have been studied and discussed.

Keywords. *Tephrosia apollinea*; *Trigonella polycerata*; *Vicia hirsuta*; *V. sativa*; ineffective nodules; root hair; nodule meristem; bacteroid zone; vascular bundle; nodule cortex.

1. Introduction

The structural features of effective nodules have been studied in detail in many leguminous plants. There is meagre information about the development and structure of ineffective nodules in legumes in general and of weeds in particular. Chen and Thornton (1940) in clover, pea and soybean; Arora (1956a,b) in *Crotalaria juncea* and *Cicer arietinum*; Gothwal (1962), Narayana and Gothwal (1964) in *Canavalia* and *Phaseolus mungo* and Srivastava (1982) in *Cassia absus* studied the structure and development of ineffective nodules. The present paper deals with the development and structure of ineffective root nodules in *Tephrosia apollinea*, *Trigonella polycerata*, *Vicia hirsuta* and *V. sativa*.

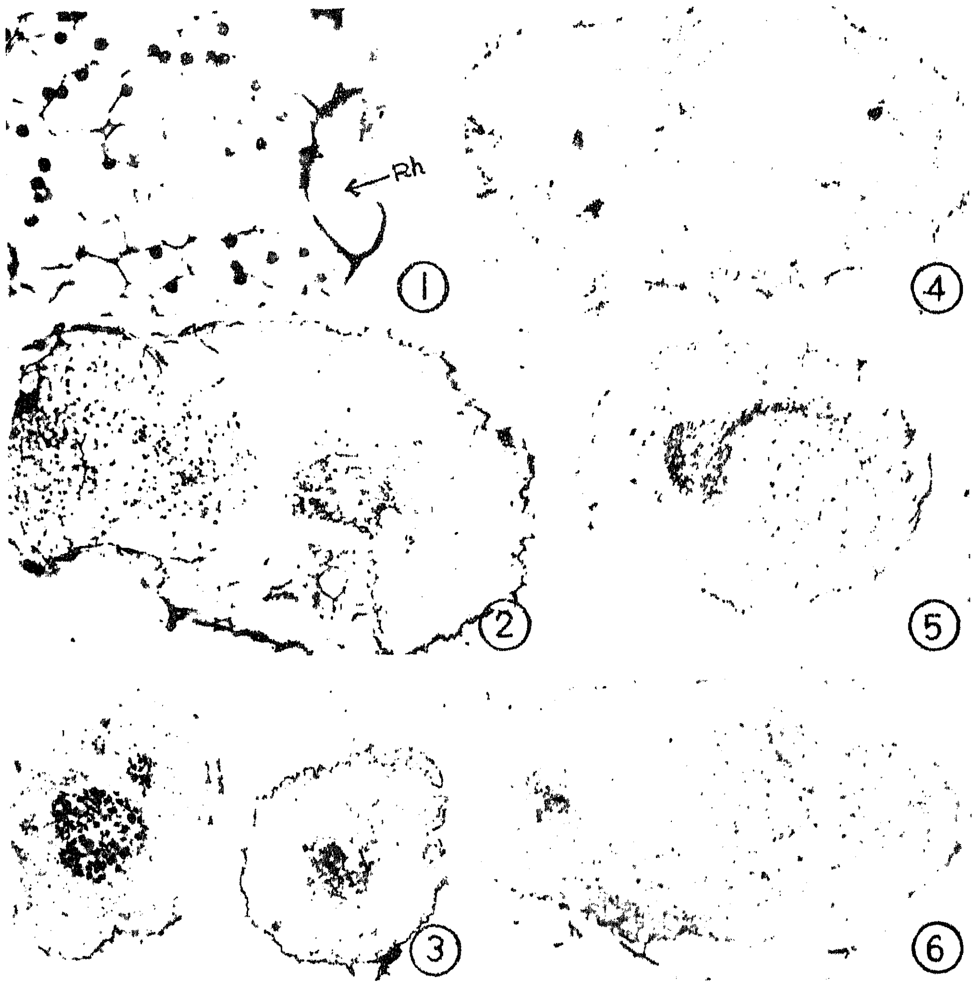
2. Materials and methods

Seedlings of *Tephrosia apollinea*, *Trigonella polycerata*, *Vicia hirsuta* and *V. sativa* were raised in culture tubes and were inoculated with the homologous strain of *Rhizobium*. After 30 days of growth the effective and ineffective nodules of different ages of these plants were collected and fixed in Navaschin's fluid for 24 hr and rinsed with 70% alcohol. After suitable dehydration and infiltration they were embedded in paraffin and sections (8–10 μ) were cut which were stained with Heidenhain's haematoxylin with erythosine as counter stain.

2.1 *Tephrosia apollinea*

Nodule was first observed on the primary root on the 10th day seedling in culture tube. The nodules were either in pairs or juxtaposed and were of two types. (i) effective nodules which were pink and (ii) ineffective nodules which were white, smaller and spherical all through.

The mode of infection in ineffective nodule was through the root hair, the base of which enlarges many times compared to its original size (figures 1, 2). There was no nodule meristem. One or two layers of root cortex enclose the central group of infected



Figures 1–6. **1.** *Tephrosia apollinea.* Ineffective nodule with an infected, enlarged root hair (Rh) (× 1300), **2.** TLS of ineffective nodule (× 125), **3.** TS of effective and ineffective nodules in close proximity (× 125), **4.** *Trigonella polycerata.* TLS of ineffective nodule (× 38), **5.** *Vicia hirsuta*, an ineffective nodule (× 40), **6.** *Vicia sativa* TLS of ineffective nodule (× 40).

cells which were traversed by infection threads. The latter were either in contact with or pass very close to the host nucleus. Neither the infected cell nucleus nor the host cell was enlarged. Generally the ineffective nodule was away from the effective nodule. In one instance an ineffective nodule was organized in the root close to the effective nodule (figure 3). The vascular supply was organised, but less extensive. This may be in keeping with the small size of the nodule.

2.2 *Trigonella polycerata*

Effective and ineffective nodules were observed in *Trigonella polycerata* on tap and secondary roots. The ineffective nodules were less in number than the effective nodules.

The mode of infection was not clear. In ineffective nodule there was no nodule meristem. The central tissue contained large cells and spaces without bacteroids. The vascular bundles of the nodule were ill-developed and the nodule cortex was thin (figure 4).

2.3 *Vicia hirsuta and V. sativa*

The ineffective nodules were smaller, spherical and fewer when compared with the effective nodules. The early development of the ineffective nodule was similar to that of the effective nodule. The presence of nodule meristem was noted but its activity was limited. The central tissue was infected but bacteroids are not formed (figure 5). The infection thread passed through many cells of the central tissue. It showed affinity towards the host nucleus. However, the latter neither got hypertrophied nor took dense stain. There was neither starch grains in this tissue nor funnel-shaped swellings of the infection thread in the region of the intercellular passage (a feature common in effective nodules of both the genera). The vascular supply was ill-developed. The nodule meristem, vascular bundles, bacteroid zone and root stele were enveloped by the common endodermal layer (figures 5, 6). In other leguminous weeds such as *Lathyrus aphaca* and *L. sativus*, the ineffective nodules have been studied and their structure was very similar to *Tephrosia apollinea*, *Vicia hirsuta* and *V. sativa* (Jain 1981).

3. Discussion

The early course of development of both effective and ineffective nodule was the same in clover, pea and soybean (Chen and Thornton 1940); *Crotalaria juncea* (Arora 1956a); *Cicer arietinum* (Arora 1956b); *Canavalia* and *Phaseolus mungo* (Gothwal 1962; Narayana and Gothwal 1964) and *Cassia absus* (Srivastava 1982). The present study clearly indicates that the early development of the ineffective nodule in *Tephrosia apollinea*, *Vicia hirsuta* and *V. sativa* was very similar to that of the effective nodule.

There was no nodule meristem in *Cicer arietinum* (Arora 1956b). The nodule meristem growth ceased after seven days and formed a small round ineffective nodule in clover, pea and soybean (Chen and Thornton 1940). In the present study there was no distinct nodule meristem in *Tephrosia apollinea*, *Trigonella polycerata*, *Vicia hirsuta* and *V. sativa*.

The poor infection and early disintegration of ineffective nodule was observed in *Crotalaria juncea* (Arora 1956a); *Cicer arietinum* (Arora 1956b). In the present study also similar observations were recorded in *Tephrosia apollinea*, *Vicia hirsuta* and *V. sativa*. Nodule cortex was usually thin in *Tephrosia apollinea*, *Trigonella polycerata*, four layered in *Vicia hirsuta* and *V. sativa*. Earlier in *Cicer arietinum*, Arora (1956b) reported that the nodule cortex was indistinguishable from the inner lying tissue. However, in *Cassia absus*, Srivastava (1982) reported that a 4–5-layered root cortex enclose a central tissue which contained very few infected hypodermal host cells embedded in thin-walled cells, containing starch grains and brown pigment. The starch grains and brown pigment were absent in the present findings and in clover, pea and soybean (Chen and Thornton 1940), *Crotalaria juncea* (Arora 1956a); and *Cicer arietinum* (Arora 1956b) in earlier findings.

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Physiological and biochemical studies on the nutritional significance of endosperm haustoria during the early stages of embryo development in *Cajanus cajan* (L.) Millsp.

P SATHIYAMOORTHY and M VIVEKANANDAN

Department of Botany, Bharathidasan University, Tiruchirapalli 620 023, India

MS received 12 August 1983; revised 7 May 1984

Abstract. Total contents of soluble starch, reducing and non-reducing sugars, soluble proteins and phenols were determined at various stages of development of embryo, endosperm and the endosperm haustoria in *Cajanus cajan* (L.) Millsp. and the results are reported here.

Keywords. Embryo; endosperm; endosperm haustoria; metabolites; hydrolases; *Cajanus cajan*.

1. Introduction

Nutrition for the developing embryo is mainly derived from the endosperm. A remarkable feature of the endosperm in some plants is the presence of haustoria that are involved in the dissolution and absorption of nutrients from the surrounding nucellus and seed coat during the early development of embryo. The development of endosperm and embryo in various Papilionaceae genera has been reviewed by Rau (1951, 1953). Johri and Garg (1956, 1959) and Seshavatharam (1982) studied the morphological aspects of haustorial development in many members of Leguminosae. The structure and function of endosperm in *Cajanus* were studied by Parikh and Sehgal (1977). In the early stages of seed development in *Cajanus*, the haustorium shows intimate association with the ovular tissue and the endosperm indicating its role in the nutrition of the growing embryo. Hitherto only cytological and cytochemical evidences have been presented for the nutritive role of the endosperm haustoria (Raghavan 1976; Torosian 1971; Bhatnagar and Kallarackal 1980). The available literature on the physiological role of the endosperm haustoria in the development of embryo, however, is rather scarce. Therefore, the present study deals with the physiology and biochemistry of endosperm haustoria in comparison with embryo and endosperm to understand the role of endosperm haustoria in the nutrition of the growing embryo. The endosperm and endosperm haustoria are shortlived and therefore the seed is exalbuminous.

2. Materials and methods

Plants of *Cajanus cajan* (L.) Millsp (pigeon pea) were grown in the University botanical garden. Flowers were tagged at the time of opening (anthesis may vary by 9 hr). Haustorial fluid was collected in embryo cup by carefully dissecting out the fresh haustorial bags at 30 ± 1°C from manually isolated endosperm in developing seeds which were collected at different time intervals of 7–11 days after flowering (DAF).

The weight of the haustoria was calculated indirectly in the following way: weight of the haustoria = total seed weight − total weight of the embryo + endosperm + seed coat (including nucellus). The weighed endosperm and embryo were microhomogenised either in distilled water or 80% ethanol or appropriate buffers. The homogenate was centrifuged at 10000 rpm at 0°C for 15 min. The supernatant was used to assay enzymes and biochemical constituents at 48 and 24 hr intervals, respectively, from 7–11 DAF. The protein content of the haustorial fluid, endosperm and embryo was determined by the method of Lowry *et al* (1951) and that of free amino acid by Lee and Takahashi (1966) method. Total soluble sugars were measured using phenol-sulphuric acid method (Dubois *et al* 1951) and the reducing sugar by Nelson's method (1944). The non-reducing sugar was calculated by deducting the reducing sugar value from that of the total sugar. The total phenol was estimated following the method of Swain and Hillis (1959). Starch was estimated by the iodine reagent.

Acid and alkaline phosphatases were assayed by the methods of Ikawa *et al* (1964) and Torriani (1967) respectively. The α and β amylases were measured using the method of Dure (1960). Cellulase was measured by the method described by Malik and Singh (1980). Protease activity was assayed by incubating the enzyme extract with casein. The TCA soluble products released by protein hydrolysis were estimated by the modified method of Penner and Ashon (1967) in combination with the method of Lowry *et al* (1951) using Folin-phenol reagent.

3. Results and discussion

The results presented in table 1 indicate changes in protein, free amino acids, total, reducing and non-reducing sugars, total phenol and starch from 7–11 days after flowering and table 2 shows changes in the enzyme activity, as the embryo and endosperm were under development and also too small prior to the 7th day of flowering. After the 11th day of flowering the endosperm haustoria show gradual depletion of their contents and the endosperm is seen as few layers surrounding the embryo.

In the embryo the amount of soluble protein shows a lot of variation from 7–11 DAF. The metabolites like free amino acids, total sugars, reducing and non-reducing sugars and total phenol show a steady decrease during the period with starch content showing a gradual increase. The activity of acid phosphatase does not show any significant difference. The peak activities of alkaline phosphatase and that of α and β-amylases were observed on the 9 DAF, whereas cellulase and protease activities decrease on the 9 and 11 DAF, respectively.

In the endosperm the total soluble protein increases up to 10 DAF after which the level decreases. The total, as well as reducing and non-reducing sugars record a steady increase. The level of total phenol increases up to 10 DAF after which it drastically decreases. The activity of acid phosphatase remains generally unaltered, whereas that of alkaline phosphatase is reduced by more than 50% on the 9 DAF. The activities of cellulase and protease increase at later stages of development. The activity of α amylase is very much pronounced in the early stage of development.

In the haustoria the levels of protein, free amino acids, total, reducing and non-reducing sugars do not conform to any pattern of development and show lot of variations. During the initial stages of development, the activities of acid and alkaline

Table 1. Changes in metabolites (μg/100 mg fresh weight) during the development of embryo, endosperm, endosperm haustoria in *Cajanus cajan*. The data are the mean values of two different experiments.

DAF*	Total protein	Total free amino acids	Total sugars	Reducing sugars	Non-reducing sugars	Total phenol	Total starch
mbryo	1389	593	556	241	315	104	20
ndosperm	805	258	290	217	23	56	—
austoria	1007	170	375	316	58	67	—
mbryo	1023	421	421	181	239	105	52
ndosperm	720	613	548	243	305	67	—
austoria	1564	687	992	506	486	56	—
mbryo	957	369	272	163	170	63	75
ndosperm	1332	782	665	270	396	71	—
austoria	1328	258	338	201	137	39	—
mbryo	1568	127	105	41	63	56	93
ndosperm	1544	1632	1032	428	604	95	—
austoria	1448	304	505	244	262	35	—
mbryo	1593	110	103	31	63	44	112
ndosperm	1580	1125	1302	449	853	35	—
austoria	1058	125	388	13	375	21	—

ys after flowering

Table 2. Changes in the activity of hydrolytic enzymes during early stages of embryo, endosperm and endosperm haustoria.

	Embryo (DAF)			Endosperm (DAF)			Haustoria (DAF)		
olytic enzymes	7	9	11	7	9	11	7	9	11
phosphatase (l NP released hr^{-1} mg $protein^{-1}$)	33	30	32	208	227	267	531	51	85
ine phosphatase (l NP released hr^{-1} mg $protein^{-1}$)	39	62	45	325	140	288	335	154	215
/lase (altose released hr^{-1} mg $protein^{-1}$)	427	773	173	1320	476	767	1193	443	300
/lase (altose released hr^{-1} mg $protein^{-1}$)	373	432	33	..	..	..	..	..	..
ase (glucose released hr^{-1} mg $protein^{-1}$)	307	159	94	249	514	487	185	343	88
ase (eptides released mg protein hr^{-1})	288	246	92	416	408	960	835	569	806

= days after flowering.
ata are the mean values of two different experiments in *Cajanus cajan*.

phatases are high and decrease in subsequent stages. However, the activity of α lase shows a drastic decrease from 7–11 DAF. The cellulase activity increases up to F and thereafter records a sharp decline. The activity of protease does not show any ge but for a sharp decline on the 9th day of analysis. The soluble starch and β-lase activity could not be detected in the endosperm and the endosperm haustoria.

In early division phase, the embryo might use metabolites provided by suspensor, degenerating synergids and antipodals (Raghavan 1976). After the 7 DAF, the embryo is dependent on the endosperm for nourishment. In the early stage, the embryo show higher content of protein which undergoes a rapid breakdown (table 1) probably due to the increased activity of protease (table 2) although it is not in conformity with increase in free amino acids. The higher content of phenol in the early stages of embryo development (table 1) may help in the process of differentiation and development (Malik *et al* 1982).

In the endosperm protein increases up to the 11 DAF and free amino acids up to the 10 DAF. The levels of total, reducing and non-reducing sugars also increase, during the course of the development of the endosperm, which serves as the nutritive tissue to the embryo by disintegration at a later stage of development (List and Steward 1965).

The endosperm haustoria are richer in protein, amino acid and sugars up to the 8 DAF than the endosperm. From these observations it is clear that in the early stage of development of the seed, (8 DAF) the endosperm haustoria play an active role in the transport of nutrients to the endosperm. That the nutrients from the nucellus and seed coat may be transported to the endosperm via haustorium has been shown by the presence of plasma membrane lined wall ingrowths in the haustorium (Torosian 1971).

High activity of phosphatases is recorded in the endosperm haustoria, compared to embryo and endosperm (table 2). In the endosperm the phosphatase activity is predominant indicating that turn over rate of phosphate ester is higher than in the embryo. The activity of α amylase has been recorded in both the endosperm and haustoria. However, the β amylase activity could not be detected. Although in the early stage of embryo both α and β-amylase activities are comparatively higher, at later stages the activity is substantially reduced. This is also probably explained by the increase in the concentration of sugars in the early stages of the embryo development (7 DAF) and also by the increasing concentration of starch grains detected in the embryo at later stages of development (11 DAF). Similarly the cellulase activity is substantially higher in the initial stages of the embryo development and at later stages the activity declines. In the endosperm the enzyme activity is maintained at the maximal level up to the period of analysis in the present study probably indicating that the endosperm supplies food to the developing embryos by degrading itself. High cellulase activity is also noticed in the endosperm haustoria but it decreases substantially at later stages (11 DAF). Similarly, the protease activity increases in the endosperm and endosperm haustoria from the very early stage of development. The increase in protease activity in the endosperm is evident by the increases in the free amino acid content as well (tables 1 and 2).

The endosperm haustoria in general are rich in hydrolytic enzymes. The activity of phosphatases is much higher in the endosperm and endosperm haustoria, compared with the embryo. Such high activities of enzymes like phosphatase are also recorded in the haustoria of *Cuscuta* (Malik and Singh 1980). Cytochemical studies on the endosperm haustoria of *Linaria bipartita* made by Bhatnagar and Kallarackal (1980) indicate that the endosperm haustoria do not provide any nutrients to the embryo directly but only through the endosperm. This is evident from table 1 that most of the metabolites in the endosperm haustoria are drastically reduced from the 9 DAF, probably due to their being gorged in the endosperm tissue. Therefore, the haustoria in addition to gathering metabolites from the neighbouring tissues also serve as a transporting channel for supply of food materials to the endosperm. The present work

confirms the recent cytochemical finding of Bhatnagar and Kallarackal (1980) that the haustoria are nutritional in function.

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Influence of repeated water stress on wheat

B K GARG, S P VYAS, S KATHJU and A N LAHIRI

Division of Soil-Water-Plant Relationship, Central Arid Zone Research Institute, Jodhpur 342 003 India

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Abstract. Fertility-induced performance alterations in wheat (cv Kalyansona) have been evaluated under two cycles of droughts at various developmental stages and also repeated droughts. The significant alleviation of growth and yield, despite the higher stress experienced by plants under improved soil fertility, seems to be related to larger root growth and greater post-drought nutrient uptake and not to favourable tissue water modulations. Limited wet-period interludes, under repeated stress, reduced these advantages. Stress-mediated increases in proline and free aminoacids and decline in chlorophyll content in leaves followed established trends. Their levels, however, were relatively higher under better soil fertility. The proline accumulation was reduced in the second cycle of drought, as compared to the first, indicating an absence of hardening effect.

Keywords. Water stress; soil fertility; wheat; nutrient uptake; metabolism.

1. Introduction

Effects of water stress on growth and yield of crops depend both on the degree of stress and the stage of growth at which it is experienced (Hsiao and Acevedo 1974; Lewis *et al* 1974; Sullivan and Eastin 1974). In this regard it has been reported (Lahiri *et al* 1973; Lahiri 1980) that adequate soil fertility substantially alleviates the adverse effects of drought on crop performance at all stages of growth. But this conclusion is essentially based on studies where a single cycle of drought was imposed, and there is hardly any evidence on the persistance of this advantage, when successive drought events occur at a specific developmental stage, within a short span of time. This paper relates some findings in this area.

2. Material and methods

Wheat (*Triticum aestivum* L cv Kalyansona) was grown in pots with 10 kg of loamy sand soil (7·1 % clay, 5·6 % silt, 63·1 % fine sand and 24·1 % coarse sand and having 0·15 % organic carbon, available phosphorus as P_2O_5 corresponding to 15 kg ha^{-1} and available potassium as K_2O corresponding to 200 kg ha^{-1}). Two plants were grown in each pot. After 10 days of sowing, these plants were subjected to moderately high (HF) or low (LF) soil fertility conditions (80 kg ha^{-1} or 20 kg ha^{-1} N and P_2O_5).

Plants under both the fertility conditions were maintained close to field capacity (*ca* 10 % soil moisture) from germination to maturity, except during drought periods indicated in table 1. Two cycles of droughts, at a given developmental stage, were imposed under D_1 through D_5 treatments while in D_6 treatment plants were subjected to consecutive droughts. Control plants were maintained close to field capacity throughout the experimental period.

Table 1. Details of drought treatments (Feekes scale (Large 1954) is adopted to indicate the developmental stages at which drought was imposed).

Drought treatments	Feekes scale	Development stage	Days from sowing			
			First drought cycle		Second drought cycle	
			Onset	Termination	Onset	Termination
D_1	2	Tillering begins	32	44	47	56
D_2	5	Leaf sheath strongly erected	47	56	60	68
D_3	8	Last leaf just visible	68	77	79	89
D_4	10	In "boot"	79	88	91	98
D_5	10·5·4	Flowering over Kernel watery ripe	91	98	101	106
D_6	Successive droughts at 32, 47, 68, 79, 91 and 101 days from sowing					

Drought was imposed by withholding of water; watering was resumed when the soil water tension under the HF treatment declined to about -15 bars (*ca* 3·2% soil moisture) associated with wilting of plants. LF-plants normally reached this stage a day or two later due to lesser vegetative growth and presumably lower water use. The second cycle of drought commenced when the leaf relative turgidity (RT %) increased to about 80% or above consequent upon watering, after terminating the first drought cycle. The same procedure was adopted for imposing repeated droughts.

Plant performance was evaluated from 10 randomly arranged pots per treatment, each having two plants. Nitrogen, phosphorus (Lindner 1944) and potassium (Jackson 1973) were estimated from representative samples of the shoot and grains at harvest. Chlorophyll, free amino acid and proline were estimated in fully expanded uppermost leaves of control and droughted plants just before terminating the first and second drought cycles of D_1, D_2 and D_3 treatments. Chlorophyll was estimated spectrophotometrically following the method of Arnon (1949). Free proline was estimated following the method of Bates *et al* (1973). The free amino acids were estimated colorimetrically, using glycine for the preparation of calibration curve, at 570 nm following the method of Yemm and Cocking (1955). Plant water status was evaluated from relative turgidity or RT % (Slatyer and McIlroy 1961) and ψ xylem by a pressure bomb (Scholander *et al* 1965).

3. Results and discussion

Results revealed (table 2) that drought, fertility and their interaction had highly significant effects on different indices of plant performance.

The marginal means for drought treatments indicate that maximum adverse effects on plant height were due to stress at D_1, D_2 and D_3 stages when the rate of elongation was fastest (Large 1954). A comparable effect was found in D_6 treatment as the plants experienced water stress at the aforesaid stages. Again, significant reduction in tiller number, with respect to the control, was due to drought at D_1 ("tillering begins") and D_2 ("leaf sheath strongly erect") which fall broadly under the tillering phase of the Feekes

Table 2. Influence of soil fertility on the performance of wheat crop under intense phasic drought.

Treatments	Plant height (cm)			Leaf number per plant			Tiller no. per plant			No. of ears per plant			Dry weight shoot (g/plant)			Dry weight root (g/plant)			Grain weight (g/plant)		
	HF	LF	Mean	HF	LF	Mean	HF	LF	Mean	HF	LF	Mean	HF	LF	Mean	HF	LF	Mean	HF	LF	Mean
control	53·1	44·9	49·0	8·0	6·0	7·0	4·0	1·8	2·9	3·3	1·0	2·2	3·71	0·89	2·30	4·63	1·76	3·19	4·28	1·02	2·65
D_1	48·4	34·1	41·2	6·4	4·6	5·5	2·2	0·3	1·3	1·6	1·0	1·3	1·33	0·44	0·88	2·03	0·48	1·25	1·81	0·68	1·25
D_2	37·3	34·2	35·8	6·8	5·3	6·1	3·3	1·2	2·2	1·3	1·0	1·2	1·56	0·75	1·15	2·58	1·14	1·86	1·37	0·80	1·08
D_3	40·1	34·5	37·3	7·8	7·0	7·4	3·6	2·3	2·9	1·7	1·0	1·4	2·51	0·96	1·73	4·35	1·61	2·98	1·24	0·74	1·99
D_4	51·0	46·7	48·9	7·9	7·4	7·7	3·6	2·5	3·0	2·3	1·0	1·7	3·48	0·99	2·23	4·53	1·75	3·14	1·24	0·68	0·96
D_5	53·1	44·9	49·0	9·4	7·1	8·3	5·0	2·0	3·5	1·9	1·0	1·5	2·95	1·11	2·03	3·25	1·10	2·18	1·92	1·09	1·50
D_6	45·5	35·0	40·2	7·1	5·0	6·1	2·9	0·3	1·6	1·2	1·0	1·1	1·22	0·38	0·80	1·24	0·45	0·84	0·58	0·44	0·51
Mean	45·9	38·2	—	7·6	6·0	—	3·5	1·5	—	1·9	1·0	—	2·39	0·79	—	3·23	1·18	—	1·78	0·78	—
LSD (%)	5		1	5		1	5		1	5		1	5		1	5		1	5		1
Fertility (F)	1·73		2·27	0·54		0·71	0·31		0·41	0·14		0·19	0·21		0·28	0·35		0·48	0·24		0·31
Drought (D)	2·99		3·93	1·01		1·33	0·58		0·77	0·38		0·50	0·40		0·52	0·65		0·90	0·45		0·59
$F \times D$	4·23		5·56	1·43		1·88	0·83		1·09	0·76		1·00	0·57		0·75	0·92		1·27	0·65		0·85

scale. The leaf number was also affected most due to water stress at these stages. Since plants in the D_6 treatment equally suffered from drought at the aforesaid stages their leaf and tiller numbers were also significantly low. However, the number of ears per plant was significantly reduced due to drought at all stages. Adverse effects of drought on the vegetative growth at the D_1, D_2, D_3 and D_6 stages led to a marked reduction of above ground dry matter production at these stages. But the effects at D_4 and D_5 stages were not large enough to be significant. The root growth was affected most by water stress at D_1, D_2 and D_6 stages. Although the grain yield was generally reduced under all drought treatments, maximum yield reduction, as compared to the control, was brought about only when drought was experienced at the flag leaf (D_4) and boot (D_5) stages. A similar effect was noticed under D_6 treatment also as these plants suffered water stress at these critical stages. Data on drought effects thus conform to the general concept that water stress at a given developmental stage inhibit its expression and the reproductive phase is most sensitive to stress as regards grain yield (Fischer 1973). It seems from the data of D_1 through D_5 stages that this trend does not substantially alter due to intense stress caused by two consecutive droughts at a given developmental stage.

Marginal means for fertility treatments (table 2) indicate a consistent and significant performance superiority of HF-plants, as compared to LF-plants.

Furthermore, the cell values (*i.e.* treatment means) interestingly reveal that irrespective of the stage (*i.e.* D_1 through D_5) at which drought was imposed, the HF-plants showed a superiority of performance in all the characters as compared to the LF-plants. The differences in most cases were statistically significant. This trend is also discernible in the D_6 treatment where plants experienced intermittent drought throughout the growing period. The data indicate that the root, as well as, shoot growth was markedly improved under the HF as compared to the LF condition under all drought treatments. In this regard it is known (Arnon 1975) that a high root:shoot ratio is a very effective means of adaptation of plants to dry conditions. The root:shoot ratio under HF condition in the control, D_1, D_2, D_3, D_4, D_5 and D_6 treatments were 1·25, 1·53, 1·65, 1·73, 1·3, 1·1 and 1·02 respectively, while under the LF condition the values were 1·98, 1·09, 1·52, 1·68, 1·77, 0·99 and 1·18 respectively. In HF condition, the root:shoot ratio increased as compared to the control when droughts were encountered during the vegetative period (*i.e.* D_1, D_2 and D_3 stages). This was, however, not discernible under LF condition. Also this ratio under stress condition was more in the HF as compared to the LF-plants particularly during the vegetative period.

The results show that the mechanism of fertility-induced drought evasion proposed (Lahiri 1980) for a single cycle of drought, does not alter under a more intense stress of two consecutive cycles of drought at different phases of development. It is tempting to speculate that the factors, such as, fertility of the soil, dynamics of the plant sensitivity to water stress during development and the period of favourable soil moisture at post-drought stages, have a greater influence on the plant performance rather than the drought event *per se* so long as plant mortality does not occur.

Table 3 indicates that absolute quantities of nitrogen, phosphorus and potassium per plant were consistently more in the HF-plants as compared to the LF-plants under all drought treatments. It seems that the favourable soil water condition which followed the drought period helped in larger uptake of nutrients in the HF-plants which in turn led to a better plant performance. The favourable soil moisture interludes being brief in D_6 treatment, uptake was not as much as in other treatments.

Stanlid (1958), in this context, pointed out that the total nutrient content per plant, rather than the nutrient concentration in the tissue, is more meaningful for understanding physiological processes. This is obvious from the data on the concentrations of nitrogen, phosphorus and potassium in the shoot tissue and in grains (table 4). The results suggest that neither drought nor fertility, caused any marked change in the nutrient concentration in the tissue. But any speculation on the basis of only concentration may be misleading as variations in dry matter production and yield caused by different treatments, substantially modulated the absolute nutrient content per plant (table 3). Again, in D_6 treatment under HF condition (table 4), when the growth

Table 3. Influence of soil fertility on the absolute levels of nitrogen, phosphorus and potassium in the shoot tissue (shoot with ear and grain) of wheat under different drought treatments, at harvest.

	Nitrogen (mg plant^{-1})		Phosphorus (mg plant^{-1})		Potassium (mg plant^{-1})	
Treatments	HF	LF	HF	LF	HF	LF
Control	107·9	28·3	7·67	4·08	141·2	32·0
D_1	47·5	19·1	6·33	2·74	45·5	15·4
D_2	36·6	22·6	5·04	3·43	41·5	24·4
D_3	43·2	24·4	6·08	3·13	73·8	27·8
D_4	64·0	27·1	7·16	2·13	91·7	20·9
D_5	76·6	22·1	7·76	3·78	67·9	31·0
D_6	31·3	12·7	3·04	1·84	44·7	11·0

Table 4. Concentrations of nitrogen, phosphorus and potassium in the shoot and grains of plants under different drought and fertility treatments.

	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
Treatments	HF	LF	HF	LF	HF	LF
Shoot						
Control	1·04	1·04	0·076	0·061	3·30	3·00
D_1	1·04	1·40	0·080	0·076	2·85	2·85
D_2	0·92	0·98	0·076	0·087	2·30	2·80
D_3	0·92	0·98	0·085	0·058	2·70	2·55
D_4	1·16	1·50	0·072	0·036	2·40	2·80
D_5	1·28	1·04	0·047	0·036	2·95	2·40
D_6	1·74	1·04	0·089	0·040	3·45	2·40
Grain						
Control	1·62	1·86	0·347	0·347	0·44	0·52
D_1	1·86	1·90	0·289	0·354	0·42	0·43
D_2	1·62	1·90	0·282	0·347	0·41	0·43
D_3	1·62	1·90	0·318	0·347	0·48	0·45
D_4	1·90	1·80	0·375	0·260	0·66	0·45
D_5	1·80	1·98	0·332	0·310	0·54	0·42
D_6	1·74	1·97	0·338	0·383	0·44	0·44

was limited by successive droughts throughout the growing period, nitrogen (but not as much of phosphorus and potassium) concentration increased in the shoot tissue because its utilisation in growth processes and dry matter production declined. A similar observation was made earlier in pearl millet crop (Lahiri and Singh 1970).

The RT % and ψxylem of the control and stressed plants (table 5), just before the termination of the first and second drought cycles at D_1, D_2 and D_3 stages, indicate that HF-plants were generally subjected to a slightly higher internal moisture stress as compared to the LF-plants in both the cycles. Thus it is likely that the alleviation of drought effect under HF conditions may be influenced more by the favourable root growth, larger uptake of nutrients during the post-drought 'wet'-phase (Lahiri *et al* 1973) and better metabolic efficiency (Kathju and Lahiri 1976). Furthermore, comparable RT % and ψxylem of the HF plants at the first and second drought cycles broadly support the earlier finding (Singh *et al* 1973) on the absence of carryover effect of one drought event to the other. The lack of any osmotic adjustment may be due to a rapid development of stress as suggested by Begg and Turner (1976) and Jones and Rawson (1979).

In the present context it is of interest to examine the consequences of repeated water stress on the levels of proline, free aminoacids and chlorophyll (table 6). The accumulation of free proline in leaves was more, under stress, in the HF-plants as compared to LF-plants. This could be due to the higher stress they experienced.

Again, the proline level consistently declined irrespective of the fertility condition at the point of maximum stress in the second cycle, as compared to the first cycle. However, Singh *et al* (1973) found, that in barley, previous exposure of the plant to water stress resulted in an increased potential for proline accumulation and explained it as a hardening effect.

This divergence could result from crop variation and differences in experimental approach. It is obvious that findings based on barley crop which have been derived through the imposition of water stress by PEG under controlled environmental condition only at the seedling stage need not necessarily be similar to those in wheat, subjected to stress by withholding of water at different growth stages, under pot culture condition in the open. The data presented here further suggest that the ability of leaves

Table 5. Relative turgidity (%) of the first fully expanded leaf and the ψ xylem (MPa) of HF and LF plants at the time when − 15 bar soil water tension was attained under the HF treatment during the first and second cycles of drought in D_1, D_2 and D_3 treatments.

		High fertility				Low fertility			
		1st cycle		2nd cycle		1st cycle		2nd cycle	
Treatments		RT	ψ xylem	RT	ψ xylem	RT	ψ xylem	RT	ψ xylem
D_1	Control	97·1	−0·8	83·5	−1·5	96·0	−0·9	83·8	−1·5
	Droughted	58·4	−2·8	62·1	−2·5	69·6	−2·2	69·5	−2·2
D_2	Control	83·5	−1·6	95·4	−0·9	83·8	−1·5	90·3	−1·2
	Droughted	66·5	−2·3	63·2	−2·5	71·4	−2·1	70·5	−2·2
D_3	Control	86·4	−1·4	82·1	−1·6	95·0	−0·9	87·0	−1·4
	Droughted	48·5	−3·2	54·3	−2·9	54·0	−2·9	54·7	−2·9

Table 6. Levels of free proline, free aminoacids (glycine eq·) and chlorophyll in the upper most expanded leaf of control and droughted plants at D_1, D_2 and D_3 stages just before watering in the first and second drought cycles.

		Free proline (mg g^{-1} dry matter)				Free aminoacids (mg g^{-1} dry matter)				Chlorophyll (mg g^{-1} dry matter)			
		HF		LF		HF		LF		HF		LF	
Treatments		1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle
D_1	Control	0·52	0·49	0·54	0·55	1·39	1·62	1·15	1·40	7·86	5·20	6·26	4·48
	Droughted	21·50	10·71	4·92	2·67	3·16	3·85	1·51	1·51	6·77	4·11	5·76	3·51
D_2	Control	0·49	0·31	0·55	0·26	1·62	1·33	1·40	1·03	5·20	5·22	4·48	4·10
	Droughted	8·47	5·22	1·75	1·35	2·42	1·58	1·57	1·08	3·77	3·45	2·78	3·05
D_3	Control	0·77	0·31	0·61	0·55	1·19	0·94	1·10	1·04	4·51	3·29	3·10	2·36
	Droughted	7·06	2·77	2·63	1·00	1·34	1·68	1·31	1·44	3·25	2·80	2·32	2·59

to accumulate proline decreased with ageing in spite of comparable plant water potential.

The free amino acid (in glycine equivalent) in the leaf similarly increased under stress, in both HF- and LF-plants, but the increase was more in the former as compared to the latter. Again, in most cases the free amino acid level declined more at the point of maximum stress, in the second cycle as compared to the first cycle, irrespective of fertility condition. This again did not suggest any hardening effect arising from the first cycle of drought.

The leaf chlorophyll content was marginally more in HF-plants, but its level declined under stress in both the fertility conditions. However, the data did not clearly indicate any hardening effect of one drought event on the other which followed subsequently.

Therefore, crop-specific influences, brought about on plants by one drought event to endure the subsequent drought, need further investigation. However, it seems that fertility-induced performance superiority of plants is maintained even if they experience consecutive water stress within a short span of time at any developmental stage. In this regard, 'wet-phase' which follows the drought event and not the severity of the drought, assumes a greater importance. However, the obvious limitations of pot-based studies, warrant a verification of the concept through field experimentation.

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inted in India.

genus *Mastigolejeunea* (Spruce) Schiffn in India

U S AWASTHI and RAM UDAR

Department of Botany, University of Lucknow, Lucknow 226 007, India

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Abstract. Studies on two species of *Mastigolejeunea* from India *viz M. humilis* (Gott) Schiffn and *M. repleta* (Tayl) St are reported. *M. humilis* exhibits considerable plasticity in morphology and sexuality as well as intracapsular spore germination. A new variety, *M. humilis* var *ciliata* var nov is described; it also occurs in Sri Lanka, Java and Thailand. *M. repleta* (Tayl) St discovered from Arunachal Pradesh for the first time, is comparatively less variable but shows interesting pattern of disposition of female inflorescences.

Keywords. Bryophyta; Jungermanniales; Lejeuneaceae; *Mastigolejeunea*.

ntroduction

recent collection of liverworts from Kerala, an area which has not so far been tigated for its bryoflora, extensive corticolous populations of *Mastigolejeunea*, ing on a variety of angiosperms from sea-level to hills, were found in several ities, revealing that the genus is neither restricted in distribution in the country nor as would appear from previous records (Mitten 1861; Stephani 1909–1912; oorn 1934; see also Udar 1976). The scanty details available for Indian taxa, which lack relevant illustrations, initiated this study.

vo species occur in India viz *M. humilis* (Gott) Schiffn from Nicobar Islands and us localities of Kerala and *M. repleta* from Arunachal Pradesh, Assam, Mt. Khasia halaya), Madras (Tamil Nadu) and Andaman Islands. The study has also revealed ccurrence of a new variety *M. humilis* var *ciliata* var nov from Kerala.

ey to the species of *Mastigolejeunea* (Spruce) Schiffn

rianth triplicate without accessory plicae.......................... *M. repleta*
rianth triplicate with accessory plicae.......................... *M. humilis*

axonomic description

Mastigolejeunea humilis (Gott) Schiffn

ngl. and Prantl, Nat. Pfl-fam. **1**, **3** 129 (1895) *Phragmicoma humilis* Gott in Gott, nb. and Nees, Synop. Hepat. 299 (1845), figures 1–59.

ants variable in size (8–25 mm long), branched irregularly by *Lejeunea*-type of ching, stem in cross-section (0·14–0·2 × 0·2 mm) with 23–35 cortical cells and al medullary cells, cells thick-walled, with prominent trigones. Leaves squarrose, ly spreading, lobe ovate or oblong, slightly falcate, 1·12–1·28 mm long,

0·64–0·88 mm wide, with rounded, obtuse or acute apex, margin entire, postical margin almost straight or incurved at about middle, revoluted along with lobule forming a continuous sac, cells arranged in diverging rows, with prominent cordate trigones and intermediate nodular thickenings, basal cells 24–44 × 12–24 μm, median cells 20–32 × 8–16 μm, marginal cells 8–16 × 4–12 μm, oil-bodies 1–7 in each cell at middle and base of leaf-lobe, ovate-elliptical (2–11 × 2–4 μm) or rounded (2–6 μm in diameter), segmented, lobule 1/2–1/3 of the lobe length, inflated along the keel, free margin and its adjacent areas appressed to the lobe, with 0–5 teeth (1–5 cells in height and width), apex of lobule hidden due to revoluted postical margin of the lobe, hyaline papilla on inner side near free margin. Underleaves obcordate (0·51–0·61 mm long, 0·56–0·77 mm wide), usually with broad median plica, apex truncate or retuse, margin entire. Monoecious. Male inflorescence terminal or intercalary, bracts hypostatic, diandrous, bract-lobe ovate (0·56–0·73 mm long, 0·36–0·48 mm wide) with acute, subacute or rounded apex and margin entire, lobule ovate (0·34–0·46 mm long, 0·18–0·22 mm wide) with acute, subacute or rounded apex and margin entire; bracteole similar to underleaf (0·33–0·35 mm long, 0·35–0·45 mm wide). Female inflorescence on short or elongated branch often repeatedly fertile, subfloral innovations 1–2, short, bract-lobe obovate or oblong (0·88–1·25 mm long, 0·56–0·75 mm wide) usually recurved, apex rounded, obtuse or acute, margin convoluted or entire, lobule ovate or oblong, apex rounded, obtuse or acute, margin entire or convoluted; bracteole similar but larger than underleaves, obovate-oblong, (0·76–1·31 mm long, 0·56–0·67 mm wide) with entire or convoluted margin; perianth obovate or oblong, triplicate often with 2–4 accessory plicae (0·96–1·76 mm long, 0·69–0·88 mm wide), rostrum small; seta with 16 peripheral and 4 central cells; capsule wall bistratose, cells of the outer layer with nodular thickenings restricted to corners but sometimes also on radial walls, cells of the inner layer with fenestrate thickenings on inner tangential wall and nodular thickenings on radial walls; spores green, variously shaped (28–52 × 20–48 μm) with minute papillae and 3–6 'rosettes' of spines; elaters 352–448 μm long, 16 μm wide, with a single spiral thickening band. Spore germination intracapsular, *Lopholejeunea*-type.

3.1a *Specimens examined*: LWU 5456/82, 5494/82 Loc.: Peechi Dam, Trichur, Kerala, Udar and party. LWU 5508/82, 5511/82, 5514/82, 5535/82, Loc.: MES Kalladi College, Mannarghat, Udar and party. LWU 5547/82, 5588/82, Loc.: Rubber Research Institute, Kottayam, Kerala, Udar and party. LWU 5594/82, 5596/82, 5599/82, 5615/82, 5622/82, 5638/82, 5645/82, Loc.: Achilatti forest, Palghat, Kerala, Udar and party. LWU 6169/82, Loc.: Murrukkadi, Kerala, Udar and party. LWU 6335/82, Loc.: Peermade, Kerala, Udar and party. LWU 6379/82, 6381/82, Loc.: Zoo, Trivandrum, Kerala, Udar and party. LWU 6388/82, 6394/82, Neyyar Dam, Trivandrum, Kerala, Udar and party. LWU 6397/82, Loc.: Ponmudi, Trivandrum, Kerala, Udar and party. NICH 310850, Loc.: In a Shorea forest south of Dharan, East Nepal. Iwatsuki (no. 23). NICH 313223, Loc.: Between Bir Gaon and Dingla, East Nepal, Iwatsuki (no. 2209). NICH 225607 b, Loc.: Java centr., Banjoemas, Bandjar, Fleischer.

3.1b *Habitat*: Corticolous.

3.1c *Associates*: *Cololejeunea himalayensis* (Pande et Misra) Schust., *C. minutissima* (Smith) Schiffn., *Frullania* sp., *Lopholejeunea abortiva* (Mitt) St., *Microlejeunea ulicina* Spruce, *Schiffneriolejeunea indica* (St) Udar et Awasthi and mosses.

3.1d *Distribution*: India (Nicobar Island and various localities of Kerala), Ambon, Banda, Borneo, Caroline Island, Celebes, Formosa, Japan, Java, Malaysia, Moluccas, Nepal, New Caledonia, New Guinea, Philippines, Ryukyu Island, Samoa, Sri Lanka, Sumatra, Tahiti and Thailand.

3.1e *Discussion*: *M. humilis* is highly variable. The leaf-lobe may be obtuse-rounded (figures 1–4, 7, 31, 35) or subacute-acute (figures 1–5, 8) in a population or all conditions may be present in the same plant but the former condition was rather common in populations forming larger lobular teeth (figures; 31, 35). The leaf-lobule exhibits a bewildering gradation in size from 1/3 to 1/2 of the lobe length and from lack of tooth (figures 12, 14) to the occurrence of 3–5 prominent teeth (figures 13, 15–17, 38, 39, 40–45) which may be up to 5 cells long and wide at base. In specimens from Neyyar Dam and Zoological Gardens, Trivandrum, the leaves on the main axis bear 3–5 teeth on their lobules which are generally 3–5 cells long and wide at base (figures 38, 39, 40–45). Similar condition was also observed in specimens from East Nepal (NICH 310850). No *Mastigolejeunea* had been known so far with more than three lobular teeth (see Schuster 1980). However, the leaves on branches exhibit a condition common in a large number of populations from Kottayam, Murrukkadi, Palghat, Peermade, Ponmudi and Trichur in Kerala where the leaf-lobule at the main axis as well as branches showed variable 0–3(4) number of teeth (figures 12–17) up to 4 cells long and wide at base (figures 14–17). There is thus an overlapping of diverse teeth number and extensive plasticity in this character.

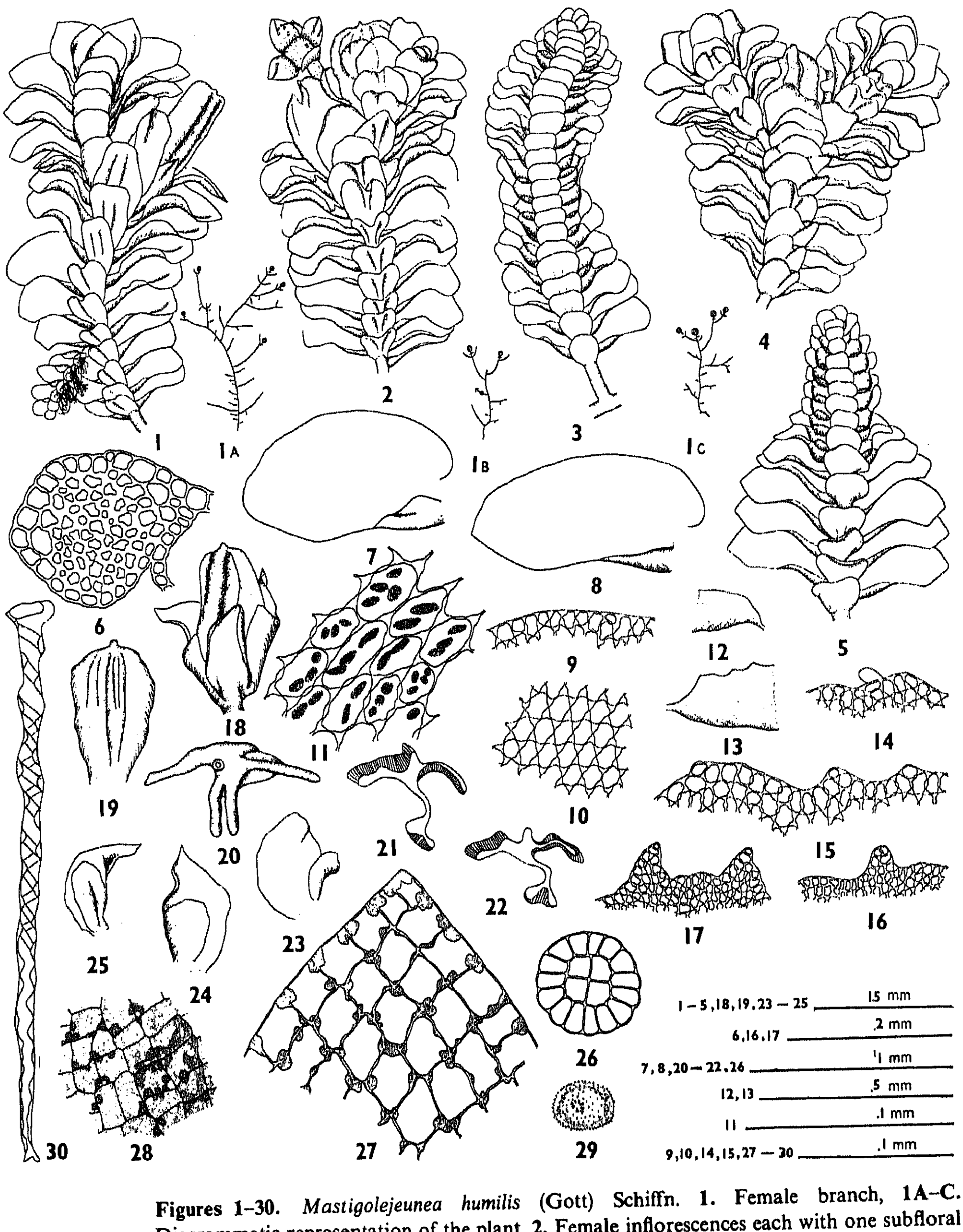
Variation occurs in the size of leaf-lobule also (1/3–1/2 of the lobe length). The plants having large lobule show larger number of teeth particularly on vegetative axis (figure 31) but the branches of the same plant bearing female inflorescence have smaller lobule (figure 32) similar to those found in populations having lesser number of lobular teeth (figures 1–5, 7, 8). Size of oil-bodies also varies markedly (figures 11, 36). The underleaves, though mostly with a wide median plica (figures 1, 2, 4, 32, 33), often lack it (figures 1, 3, 31). Sexual expression also does not appear to be stable in this species. There are monoecious plants as well as dioecious plants in a population or isolated populations consist of either male or female plants. The perianth also shows some plasticity particularly in shape. Commonly it is obovate but occasionally it may be oblong with lateral margins parallel for most of their length (figures 1, 18).

Intracapsular spore germination in this species is reported for the first time (figures 53–59). A few celled globose protonema (*P*) develops within the exospore (figure 58) from which later a leafy shoot, which bears ovate primary leaf (*A*) (figure 58) saccate-inflated juvenile leaf (*J*) (figure 59) and a narrow, ovate underleaf (*U*) (figure 59) is formed.

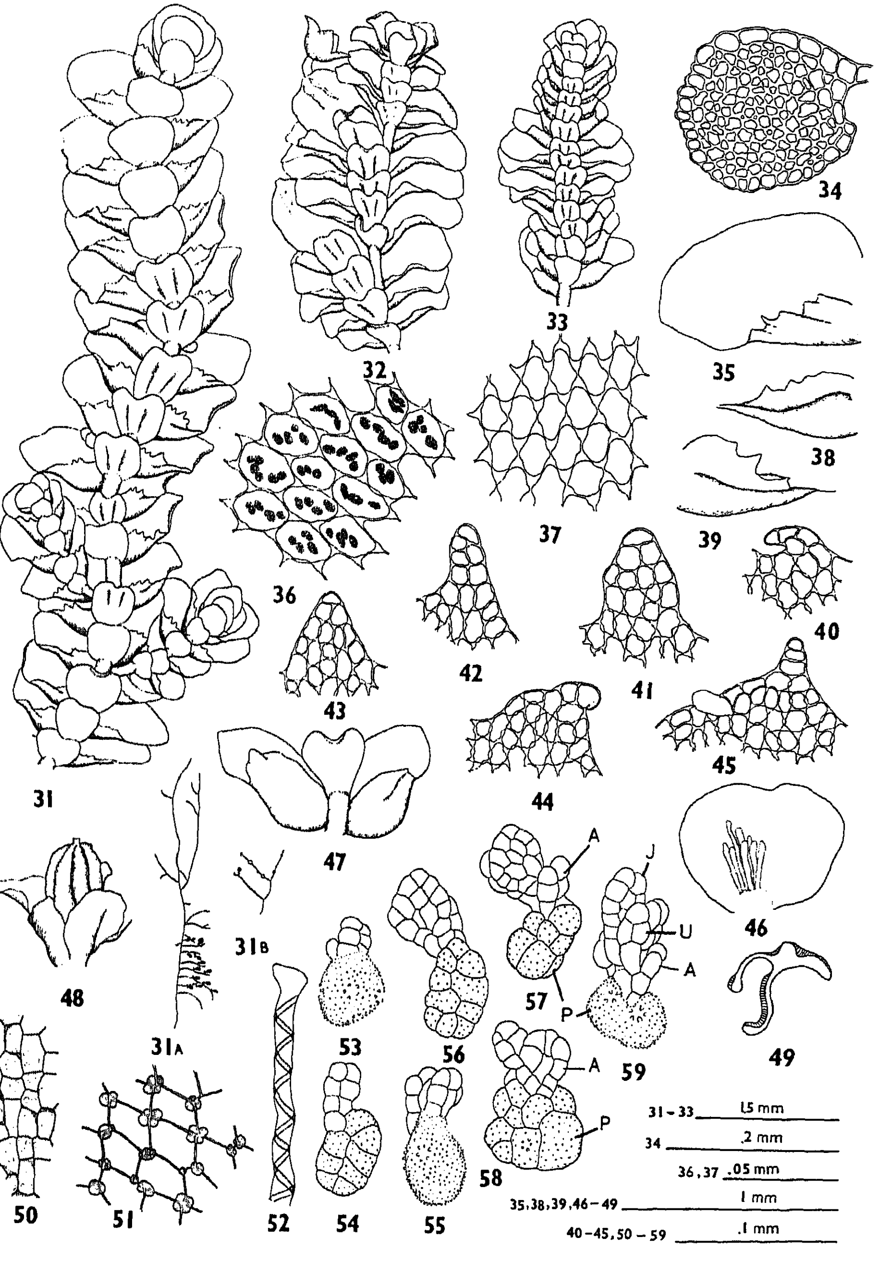
3.2 *Mastigolejeunea humilis var ciliata var nov*, figures 60–73

Haec variety a *Mastigolejeunea humilis* in lobuli folii e 1 denticulis, dente uniseriato, 5–6 cellulae longae differt.

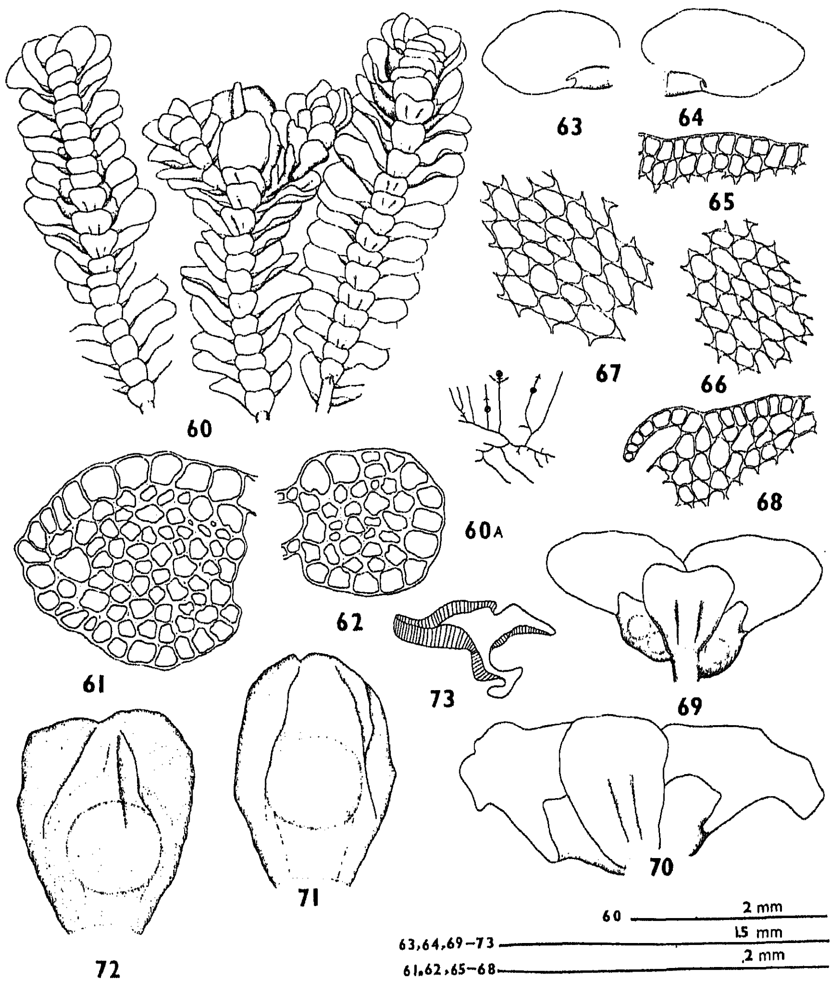
Plants smaller (10–15 mm long), branched irregularly by *Lejeunea*-type of branching, stem in cross-section (0·10–0·14 mm in diameter) with 16–24 cortical and several medullary cells, cells thick-walled, with prominent trigones. Leaves less squarrose, widely spreading, lobe oblong, slightly falcate, (1·28–1·68 mm long, 0·56–0·96 mm wide) with rounded, obtuse or subacute apex, margin entire, postical margin incurved, often



Figures 1–30. *Mastigolejeunea humilis* (Gott) Schiffn. **1.** Female branch, **1A–C.** Diagrammatic representation of the plant, **2.** Female inflorescences each with one subfloral innovation, **3.** Intercalary male inflorescence, **4.** Two subfloral innovations, **5.** Terminal male inflorescence, **6.** Cross-section of the stem, **7,8.** Leaves, **9.** Marginal cells of the leaf-lobe, **10.** Median cells of the leaf-lobe, **11.** Basal cells of the leaf-lobe with oil-bodies, **12.** Leaf-lobule without teeth, **13.** Leaf-lobule with distinct teeth, **14–17.** Portions of the leaf-lobule showing teeth, **18.** Female bracts, bracteole and perianth, **19.** Perianth showing accessory plicae, **20.** Perianth in top view, **21, 22.** Cross-section of the perianth, **23–25.** Female bracts, **26.** Cross-section of seta, **27.** Outer layer of capsule wall, **28.** Inner layer of the capsule wall, **29.** Spore, **30.** Elater.



Figures 31–59. *Mastigolejeunea humilis* (Gott) Schiffn. **31.** Portion of the plant, **31A, B.** Diagrammatic representation of the plants, **32.** Female inflorescence each with one subfloral innovation, **33.** Male inflorescence, **34.** Cross-section of the stem, **35.** Leaf, **36.** Basal cells of the leaf-lobe with oil bodies, **37.** Median cells of the leaf-lobe, **38, 39.** Leaf-lobule showing marginal teeth, **40–44.** Teeth of leaf-lobule, **45.** Portion of leaf-lobule showing marginal tooth and hyaline papilla, **46.** Underleaf, **47.** Male bracts and bracteole, **48.** Female bracts and perianth, **49.** Cross-section of the perianth, **50.** Innerlayer of the capsule wall, **51.** Outerlayer of the capsule wall, **52.** Elater, **53–59.** Stages of spore germination. (*P* = protonema; *A* = primary leaf; *J* = juvenile leaf; *U* = underleaf).



Figures 60–73. *Mastigolejeunea humilis* var *ciliata* var nov **60.** Female inflorescence with two subfloral innovations and terminal and intercalary male inflorescence, **60A.** Diagrammatic representation of the plant, **61, 62.** Cross-section of the stem, **63, 64.** Leaves, **65.** Marginal cells of the leaf-lobe, **66.** Median cells of the leaf-lobe, **67.** Basal cells of the leaf-lobe, **68.** Portion of the leaf-lobule showing teeth, **69.** Male bracts and bracteole, **70.** Female bracts and bracteole, **71, 72.** Perianth, **73.** Cross-section of the perianth.

revoluted along with lobule forming a continuous sac, cells arranged in diverging rows, with prominent cordate trigones and intermediate nodular thickenings, basal cells 20–24 × 8–16 μm, median cells 12–20 × 8–12 μm, marginal cells 8–12 × 8–12 μm, lobule almost rectangular, *ca* 1/3 of the lobe length (0·22–0·26 mm long, 0·11–0·16 mm wide), inflated along the keel, free margin with a single uniseriate curved tooth of (2–) 5–6 cells in length, apex truncate, hyaline papilla on inner side near the free margin. Underleaves obcordate (usually 0·40–0·45 mm long, 0·35–0·43 mm wide), raised along the median line forming a wide plica or flat, margin entire, apex retuse or truncate, rhizoid if present lie at the base. Monoecious. Male inflorescence on lateral branch, either intercalary or

terminal, bracts hypostatic, diandrous, lobe ovate (0·64–0·67 mm long, 0·35–0·41 mm wide), with rounded, obtuse or subacute apex and entire margin, lobule ovate (0·32–0·40 mm long, 0·16–0·21 mm wide), with rounded, obtuse or subacute apex and entire margin; bracteole similar to underleaves (0·41–0·48 mm long, 0·40–0·45 mm wide). Female inflorescence terminal on an elongated branch, with usually 2 subfloral innovations, bract-lobe ovate-oblong, recurved, larger than leaf-lobe (0·67–1·02 mm long, 0·57–0·80 mm wide), with rounded or obtuse apex and entire margin, lobule 1/2 or less than 1/2 of the lobe length (0·35–0·57 mm long, 0·32–0·35 mm wide) with rounded or obtuse apex, slightly extending beyond the keel; bracteole similar but larger than underleaves, *ca* 2/3 of the perianth length (0·72–0·80 mm long, 0·60–0·64 mm wide), perianth obovate (1·08–1·12 mm long, 0·80–0·83 mm wide), tri-plicate, with 1–3 accessory plicae, plicae smooth, rostrum inconspicuous. Rest not seen.

3.2a *Specimens examined*: LWU 6226/82 (Holotype), Loc.: Kumily, Kerala, Udar and party, LWU 5893/82, Loc.: Munnar, Kerala, Udar and party, LWU 6203/82, 6207/82, 6210/82, Loc.: Thekkady, Kerala, Udar and party.
NICH 225607a, Loc.: Java Occ. Res. Batavia, Buitenzorg, Schiffner (257, Hep. Sel. et Crit.).
NICH 225608, Loc.: Ceylon Centr. Peradenya, Schiffner (254, Hep. Sel. et Crit.).
NICH 378949, Loc.: Wang chong-arg, Sakaerat, Nakon, Rachsina; Thailand, Mernnosee (no. 616).

3.2b *Habitat*: Corticolous.

3.2c *Associates*: *Frullania* sp.

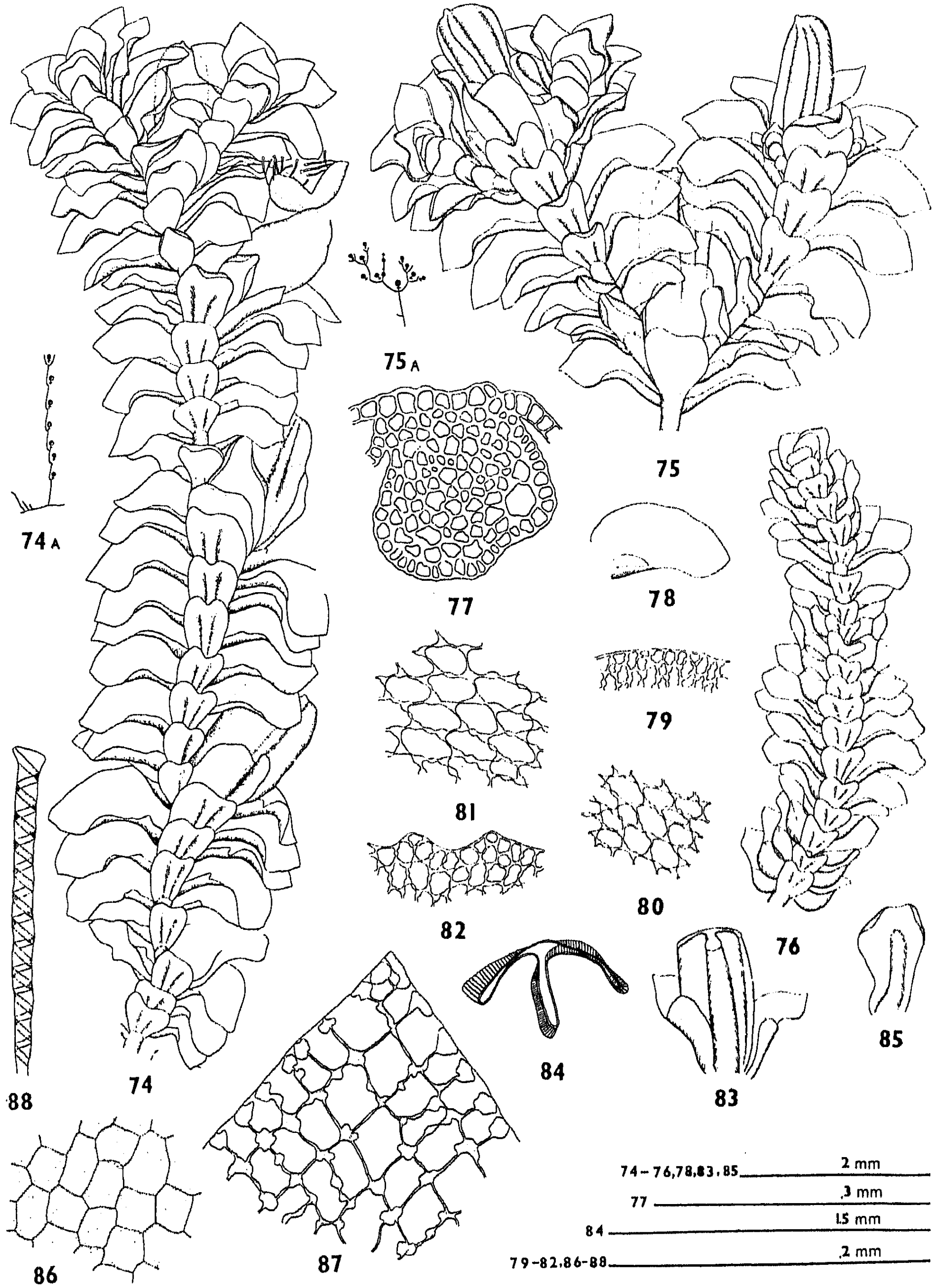
3.2d *Distribution*: India (Kerala), Java, Sri Lanka (Ceylon Centr.) and Thailand.

3.2e *Discussion*: Specimens from Sri Lanka, Java and Thailand cited above were labelled *Mastigolejeunea humilis* (Gott) Spr. However, in all these specimens the leaf-lobule had a (2–) 5–6 cells long, uniseriate apical tooth (sometimes a small preapical tooth also present) similar to that found in the Indian populations. Kitagawa (1971) also observed a similar condition in specimens from Penang, Malaysia. The variety is thus not confined to India but has a much wider distribution. The apex of the leaf-lobe which is highly variable in *M. humilis* is more or less stabilized in the present variety as it mostly has a uniseriate apical tooth (figures 60, 63, 64, 68) in Indian specimens as well as those from Sri Lanka, Java and Thailand.

3.3 *Mastigolejeunea repleta (Tayl.) St*

Spec. Hepat. IV: 772 (1912). *Lejeunea repleta* Tayl., L. J. of Bot. V: 392 (1846). *Lejeunea wardiana* Mitt. J. Proc. Linn. Soc. (London) 5: 109 (1861) = *Brachiolejeunea wardiana* (Mitt.) St. Spec. Hepat. V: 129 (1912). *Mastigolejeunea wightii* St. Spec. Hepat. IV: 773 (1912), figures; 74–88.

Plants robust (10–30 mm long), branched irregularly by *Lejeunea*-type of branching, stem in cross-section (0·22–0·24 × 0·20–0·22 mm) with 30–35 (–40) cortical and several medullary cells, cells thick-walled, with prominent trigones. Leaves squarrose, widely spreading, lobe oblong, slightly falcate (1·28–1·68 mm long, 0·56–0·96 mm wide), with



Figures 74–88. *Mastigolejeunea repleta* (Tayl) St. **74.** Female inflorescences in a series mostly with one subfloral innovations, **74A.** Diagrammatic representation of the plant, **75.** Female inflorescences each with two subfloral innovations, **75A.** Diagrammatic representation of the plant, **76.** Male inflorescence, 77. Cross-section of the stem, **78.** Leaf, **79.** Marginal cells of the leaf-lobe, **80.** Median cells of the leaf-lobe, **81.** Basal cells of the leaf-lobe, **82.** Portion of leaf-lobule showing marginal teeth, **83.** Female bracts and perianth with apex cut off, **84.** Cross-section of the perianth, **85.** Female bracteole, **86.** Innerlayer of the capsule wall, **87.** Outer layer of the capsule wall, **88.** Elater.

acute apex, margin entire, postical margin incurved, often revoluted along with lobule forming a continuous sac, cells arranged in diverging rows, with prominent cordate trigones and intermediate nodular thickenings, basal cells 28–44 × 12–20 μm, median cells 16–28 × 8–16 μm, marginal cells 12–16 × 3–12 μm, lobule almost rectangular, *ca* 1/3 of the lobe length, (*ca* 0·48 mm long, 0·16–0·27 mm wide), often with a fold, with 1–2 teeth, apex hidden due to revoluted postical margin of the lobe. Underleaves obcordate, longer than wide (0·53–0·80 mm long, 0·51–0·73 mm wide), usually raised along the median line up to apex forming a wide plica, margin entire, apex retuse or truncate, rhizoids if present lie at base. Monoecious. Male inflorescence on lateral branch or as subfloral innovation, bracts hypostatic, lobe ovate (0·90–1·20 mm long, 0·50–0·80 mm wide), with acute apex and entire margin, lobule ovate, (0·30–0·40 mm long, 0·25–0·30 mm wide), with obtuse or subacute apex, margin entire; bracteoles similar to underleaves, slightly smaller or equal in size (0·51–0·70 mm long, 0·50–0·60 mm wide). Female inflorescence terminal on an elongated branch, with 1–2 subfloral innovations, bract-lobe ovate-oblong, smaller than leaf-lobe (1·23–1·31 mm long, 0·32–0·48 mm wide), with acute apex and entire margin, lobule obovate or rectangular, 1/2 or slightly more than 1/2 of the lobe length (0·90–1 mm long, 0·16–0·25 mm wide), sometimes slightly extending beyond the keel, apex rounded, margin entire; bracteole similar but longer than underleaves, slightly less than 1/2 of the perianth length (0·90–1 mm long, 0·70–0·80 mm wide), perianth oblong, (2·11–2·16 mm long, 0·72–0·91 mm wide), tri-plicate, plicae broad and smooth, rostrum small, seta with 16 peripheral and 4 central cells, capsule wall bistratose, cells of the outer layer with nodular thickenings at corners and radial walls, cells of the inner layer with plurifenestrate thickenings on inner tangential wall and nodular thickenings on radial walls; spores green, variously shaped (25–46 × 23–41 μm), with minute papillae and 3–6 'rosettes' of spines; elaters 312–400 μm long and 16 μm wide, single spiral thickening band.

3.3a *Specimens examined*: G 1924 (Holotype) *Mastigolejeunea repleta* (Tayl) St., Loc.: Madras, Wight G 14988 (Holotype), *Mastigolejeunea indica* St., Loc.: Nicobar Island, E. H. Man. G 14990, *Mastigolejeunea indica* St., Loc.: Andaman Island, E. H. Man. G 12507, *Mastigolejeunea wightii* St., Loc.: India orientalis, Wight. G 19972, *Brachiolejeunea wardiana* (Mitt.) St. = *Lejeunea wardiana* Mitt., Loc.: Bhutan, Griffith. LWU 6837/82, Loc.: Drizing Pam forest (New Bomdila) Kameng, Arunachal Pradesh, eastern India, D. K. Singh.

3.3b *Habitat*: Corticolous.

3.3c *Associates*: *Brachiolejeunea sandvicensis* (Gott) Schust., *Plagiochila sp.*, *Porella densifolia* (St) Hatt. and *Lophozia* sp.

3.3d *Distribution*: India (Arunachal Pradesh, Assam, Khasia Mt., Madras, Andaman Island), Bhutan, Burma, Hong-Kong and Philippines.

3.3e *Discussion*: Verdoorn (1934) treats *Mastigolejeunea indica* St., *Brachiolejeunea wardiana* (Mitt) St and *Mastigolejeunea wightii* St as synonyms of this species. However, the type of *M. indica* (G 14988) which we have examined is akin to *Schifferiolejeunea pulopenangensis* (Gott) Gradst. rather than *Mastigolejeunea* (see also Kitagawa 1973). *M. repleta* (Tayl) St differs from *M. humilis* in having female bracts

smaller than leaves (figure 75) and the triplicate perianth lacking accessory folds (figures 83, 84). The type of *M. repleta* (G 1924) and the specimens from Bhutan *Brachiolejeunea wardiana* (G 19972), examined by us, show repeatedly floriferous branches as each female inflorescence has two subfloral innovations which are again floriferous (figures 75, 75A). In the specimens from Arunachal Pradesh (LWU 6837/82) apart from the above conditions, some plants showed 4–5 inflorescences in a series all on one side of the axis (figure 74) with each inflorescence giving rise to abbreviated subfloral innovations. This condition is common in a *Archilejeunea* (Spruce) Schiffn (Udar and Awasthi 1981, 1981a, 1982) but is rather rare in *Mastigolejeunea.*

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A contribution to the embryology of *Trachelospermum fragrans* Hook. f. (Apocynaceae)

K C SUD
Department of Botany, Hans Raj College, Delhi 110007, India

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Abstract. Embryology of *Trachelospermum fragrans* Hook. f. is described. The anther wall consists of the epidermis, fibrous endothecium, 2–3 middle layers and parietal tapetum. Rarely, endothecium shows 2 layers. The tapetum is uniseriate and remains uninucleate throughout. Simultaneous cytokinesis in pollen mother cells produces tetrahedral and isobilateral tetrads. Degeneration of few pollen grains is recorded. Pollen grain is triporate and 3-celled with few starch grains at anthesis. The ovule is hemianatropous, rarely anatropous, unitegmic and tenuinucellate. The chalazal megaspore of the linear tetrad develops into the 8-nucleate embryo sac of the Polygonum type. Synergids are hooked and the ephemeral antipodals appear egg-like. Fertilization is porogamous. Triple fusion occurs a little earlier than syngamy. The endosperm *ab initio* is free nuclear and later becomes cellular. Endosperm haustorium is present. Embryogeny conforms to the Solanad type.

Keywords. *Trachelospermum fragrans*; embryology; Apocynaceae.

1. Introduction

The embryological work on Apocynaceae was reviewed by Schnarf (1931), and noteworthy contributions to the embryological studies of the family were made by Fyre and Blodgett (1905), Guignard (1917a, b), Andersson (1931), Meyer (1938) and Rau (1940). While summarising the embryological data on Apocynaceae, Davis (1966) mentioned the controversy concerning the nature of division of pollen mother cells. Similarly, due to the occurrence of different types of embryogeny, Maheshwari Devi (1971) called Apocynaceae 'a heterogenous group' and pointed out the need for further investigations on this aspect. Subsequently, only a few contributions to the embryological literature of the family have been made by Murty and Chauhan (1966), Maheshwari Devi (1970, 1971, 1974), Bhasin (1971) and Lamba (1974, 1976). In view of the scant existing information on the embryology of the family Apocynaceae, the present findings on *Trachelospermum fragrans* Hook. f. are of interest.

2. Material and methods

The study material was collected from Simla hills and fixed in formalin–acetic alcohol. Tertiary-butyl alcohol series was used for dehydration before embedding the material in paraffin wax (m.p. 56–58°C). Serial sections (8–14 μ), were stained in Delafield haematoxylin as well as safranin-fast green combination of which the first gave the best results.

3. Observations

3.1 *Microsporogenesis and male gametophyte*

Anther is bithecous. In young anther, the cells of the hypodermal archesporium undergo periclinal divisions to produce an outer layer of primary parietal cells and an inner layer of sporogenous cells (figures 1, 2). Further periclinal divisions in the parietal layer result in the formation of a fibrous endothecial layer, 2–3 middle layers and a single layered tapetum (figures 3, 4). The tapetum is of secretory type. The tapetal cells remain uninucleate throughout and possess 1 or 2 prominent vacuoles (figure 4). The anther wall is thus constituted by an epidermis, a fibrous endothecium, 2–3 middle layers and a tapetum (figure 4). Rarely, the fibrous endothecium appears 2-layered (figure 5). As wall layers mature, epidermal cells appear more prominent. However, the endothecium becomes inconspicuous in the old anther. Disorganisation of the middle layers and the tapetum starts at the tetrad stage of the microspores. A few microspores also appear disintegrating soon after cytokinesis (figure 6).

Divisions of the sporogenous tissue result in the formation of pollen mother cells which undergo meiotic divisions (figure 7) followed by simultaneous cytokinesis to produce tetrahedral (figure 8) and isobilateral (figure 9) tetrads. Young pollen grains are globular or somewhat spherical in outline with vacuolated cytoplasm. Before division the nucleus is seen shifting toward periphery. Division of the nucleus is followed by a lenticular wall to form the generative and vegetative cells (figure 10). Subsequently, the lenticular wall disappears. Mature pollen grains are triporate and 3-celled during shedding (figure 11). The exine is quite smooth. Only a few starch grains are recorded in a fully-developed pollen grain.

3.2 *Megasporogenesis and female gametophyte*

The ovary is superior and bicarpellary with 3–many ovules in each carpel. The ovule is hemianatropous, rarely anatropous, unitegmic and tenuinucellate. The rapid elongation of the integument makes the 8-nucleate embryo sac deep-seated. The hypodermal archesporial cell (figure 12) enlarges considerably to function directly as the megaspore mother cell (figure 13). It undergoes meiotic divisions and produces a linear tetrad of megaspores (figure 14). Rarely, T-shaped tetrads (figure 15) are also observed. The upper three megaspores degenerate while the chalazal one becomes functional (figures 14, 15).

With the enlargement of the chalazal megaspore its nucleus undergoes three successive mitotic divisions to produce 2, 4 and 8-nucleate embryo sacs (figures 16–18). It is at the two-nucleate stage that there occurs the disorganization of the epidermal layer of the nucellus surrounding the embryo sac. A distinct integumentary tapetum has not been observed. Mature embryo sac (figure 18) is broader in the middle, and narrow and slightly bent at the chalazal end. The uninucleate synergids are pear-shaped, hooked and with a prominent basal vacuole. Antipodals are elongated and somewhat egg-like, uninucleate and ephemeral but their remnents persist till the formation of the secondary nucleus. Rarely, 8-nucleate embryo sac is seen showing 3-micropylar and 5-chalazal free nuclei (figure 19). Mature embryo sac contains a considerable amount of starch grains which are consumed after fertilization.

3.3 *Pollination and fertilization*

The pollen tube enters the embryo sac through the micropyle. One male gamete lies near the egg and the other near the polar nuclei (figure 20). Later, the polar nuclei come to lie adjacent to the egg apparatus. The male nucleus fuses with polar nuclei just before the other male nucleus fuses with the egg.

3.4 *Endosperm*

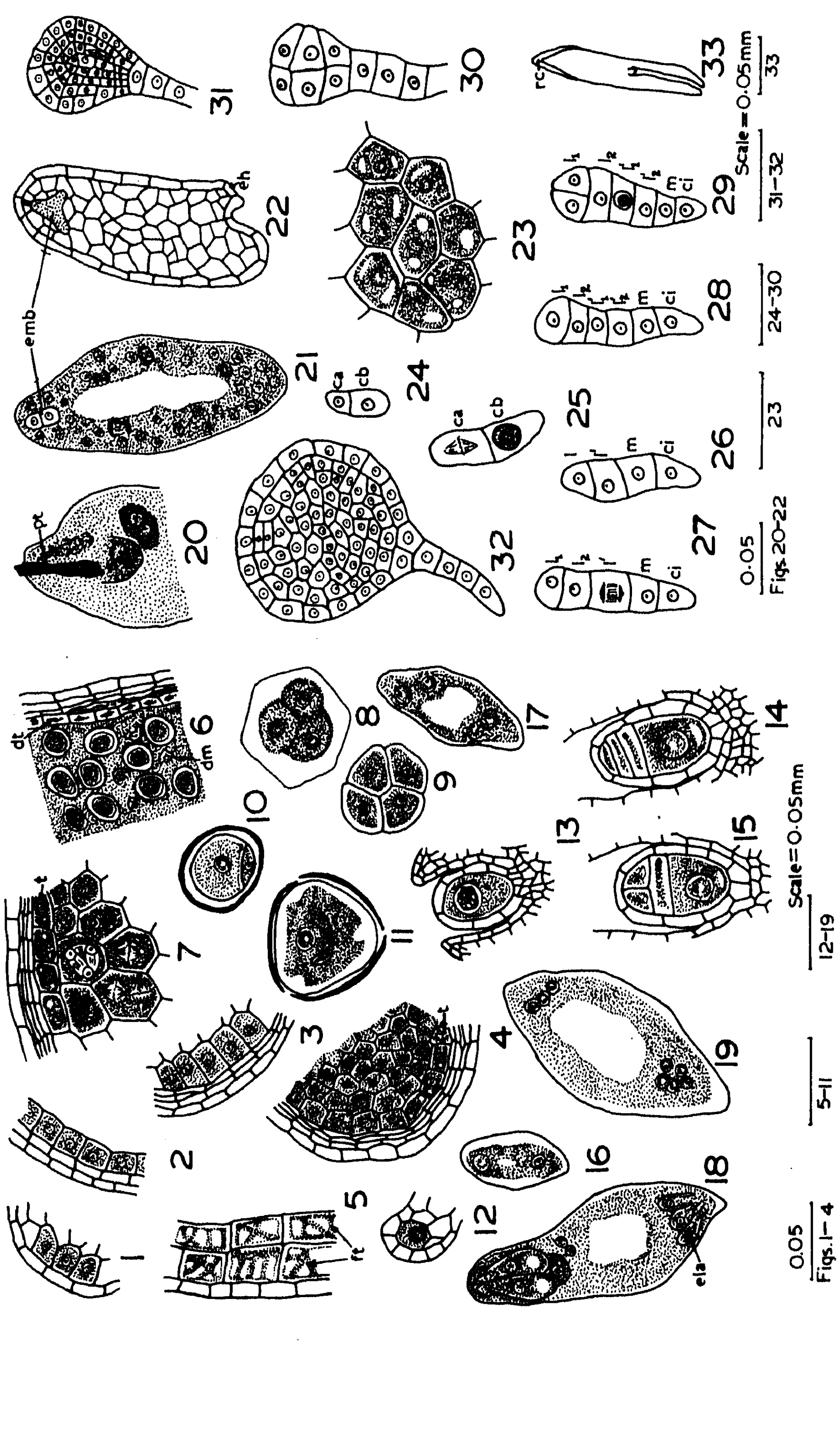
The fertilized embryo sac grows considerably in size. The primary endosperm nucleus moves to the centre where it undergoes free nuclear divisions. Due to the formation of a central vacuole, the nuclei are shifted towards the periphery of the embryo sac (figure 21). Cell formation in the endosperm begins from periphery at the 4–6-celled proembryo stage. By the time a heart-shaped embryo is formed, the entire embryo sac becomes cellular (figure 22). A few endosperm cells appear 2-nucleate (figure 23). Endosperm gets consumed by the developing embryo. The seeds are ex-albuminous. Endosperm haustoria (figure 22) are seen developing at the chalazal end of the embryo sac.

3.5 *Embryo*

A transverse division of the enlarged fertilized egg results in the formation of a terminal cell, *ca* and a basal cell, *cb* (figure 24). The second transverse division in both the cells results in a linear proembryo of 4-cells, l, l', m and ci (figures 25, 26). Another transverse division in each of the two cells l and l' produces 4 cells termed l_1, l_2, l'_1 and l'_2 (figures 27, 28). Due to the two vertical divisions, which are at right angles to one another, in the cells l_1, l_2 and l'_1, quadrants are formed (figures 29, 30) which undergo divisions in all directions to form the embryo proper (figures 31, 32). The tier l_1 eventually gives rise to two leafy cotyledons and the tier l_2 to the hypocotyledonary region. The derivatives of the tier l'_1 contribute to the root proper. Subsequent transverse divisions in one or more cells of the l'_2, m and ci result in the formation of a single-layered suspensor of 4–7 cells (figure 32). The development of the embryo is thus of the Solanad type. Mature embryo shows well-defined root cap, hypocotyl, root-axis, plumule and two cotyledons (figure 33).

4. Discussion

The development of the anther wall in *Trachelospermum fragrans* conforms to the Dicotyledonous type (Davis 1966). The present study shows 1–2 layered fibrous endothecium and 2–3 middle layers. This is contrary to Maheshwari Devi (1971) who reported a multiple endothecium and 10–14 middle layers in *Voacanga foetida* Rolfe. The endothecium becomes inconspicuous in the old anther. A similar condition was reported in *Cerbera odollam* Gaertn and *Vallaris heyneii* Spreng by Rau (1940). An uniseriate parietal tapetum has been found in this plant as in *Thevetia nerifolia* Juss and *Alstonia scholaris* R. Br (Meyer 1938), *Cerbera odollam* (Rau 1940), *Lochnera pusilla* K. Schum (Murty and Chauhan 1966), *Catharanthus roseus* (Linn.) G. Don, *C. pusillus* (Murr.) G. Don, *Holarrhena antidysenterica* Wall., *Rauvolfia serpentina* L. Benth. ex Kurz, *Voacanga foetida* and *Carissa spinarum* Linn. (Maheshwari Devi 1971, 1974). The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Figs. 1–4
5–11
12–19
Scale = 0·05mm
0.05
Figs. 20–22
23
24–30
31–32
33
Scale = 0·05mm

Figures 1–33. Stages in the development of male and female gametophytes, endosperm and embryo in *T. fragrans* Hook. f. **1.** archesporial cells; **2.** primary parietal and primary sporogenous layers. **3.** wall layers and sporogenous layer; **4.** epidermis, endothecium, middle layers, tapetum and pollen mother cells; **5.** a portion of the 2-layered fibrous endothecium; **6.** young microspores, the disorganising microspores and tapetum; **7.** microspore mother cells in meiotic division; **8, 9.** tetrahedral and isobilateral tetrads of microspores; **10.** 2-celled young pollen grain; **11.** 3-celled mature pollen grain; **12.** LS ovule showing archesporial cell; **13.** LS part of nucellus showing megaspore mother cell; **14, 15.** linear and *T*-shaped tetrads of megaspores, note the functional chalazal megaspore; **16, 17.** 2 and 4-nucleate embryo sacs, respectively; **18.** organised 8-nucleate embryo sac; **19.** abnormal embryo sac showing 3-micropylar and 5-chalazal nuclei; **20.** upper portion of embryo sac showing triple fusion and syngamy; **21.** LS of embryo sac showing nuclear endosperm; **22.** LS of embryo sac showing cellular endosperm, heart-shaped embryo and endosperm haustorium; **23.** a portion of endosperm showing 2-nucleate cells; **24–32.** stages in embryogeny; **33.** LS of mature embryo.

Abbreviations. *t*, tapetum; *ft*, fibrous thickenings; *dm*, disintegrating microspore; *dt*, disorganising tapetum; *ela*, egg-like antipodals; *pt*, pollen tube; *emb*, embryo; *eh*, endosperm haustorium; *rc*, root cap.

present study shows simultaneous cytokinesis of pollen mother cells. Th
corroborates with earlier findings of Andersson (1931), Meyer (1938), F
Maheshwari Devi (1971, 1974). As usual for Apocynaceae, pollen grains
3-celled stage. However, anthesis at the 2-celled stage has been
Catharanthus pusillus by Bhasin (1971) and *Rauvolfia serpentina* by La
few microspores have been seen disorganising soon after cytokinesis, p
to the failure of the tapetum to provide full nourishment to all t
microspores in a sporangium.

As in all the other investigated members of Apocynaceae, the ar
functions directly as the megaspore mother cell and forms a linear
occurrence of *T*-shaped tetrads in this plant resembles with the ear
Maheshwari Devi (1970, 1971) in *Catharanthus roseus*. As usual for t
chalazal megaspore develops into 8-nucleate embryo sac of the Polyg
well-defined integumentary tapetum has been reported in *Amsonia tab*
Walt and *Rhazya orientalis* Decne by Andersson (1931), but in the pre
distinct tapetum has not been observed which confirms the earlier obser
(1940) and Lamba (1974) on some other Apocynaceae. Abnormal emb
micropylar and 5 chalazal free nuclei, egg-like antipodals and hoo
constitute the important features of *T. fragrans*. Maheshwari Devi (
reported hooked synergids in *R. serpentina*. Disorganisation of epiderm
nucellus surrounding the embryo sac starts at the 2-celled stage of em
other investigated taxa of Apocynaceae (Rau 1940; Lamba 1974).

In *C. roseus* triple fusion occurs simultaneously with fertilization (Ma
1971). The present investigation shows that triple fusion takes place a lit
syngamy as in *Vallaris heyneii* (Rau 1940) and *Lochnera pusilla* (Murty
1966).

Endosperm development is of the nuclear type as reported by Murty
(1966), and Maheshwari Devi (1971, 1974) in some other Apocynaceae. T
endosperm haustoria in Apocynaceae was first recorded in *Lochnera pu*
and Chauhan (1966), later by Maheshwari Devi (1971, 1974) in *C. roseus*,
Carissa spinarum and is now being reported in *T. fragrans*. The developm
in this plant conforms to the Solanad type. *Trachelospermum fragran*
resembles the hitherto investigated members of Apocynaceae.

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Contributions to our knowledge of Indian algae-III. Euglenine
Part 2.

M T PHILIPOSE

Nenagh Cottage, Coonoor 643 101, India

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Abstract. An illustrated account of six species and ten varieties of the genus *Lepocin* and thirty-nine species and ten varieties of the genus *Phacus* Duj from inland waters east, central and south India collected during 1937–76 together with their distribut Indian region is given.

Of these, three varieties of *Lepocinclis* (*L. lefevrei* var *cuttackensis*, *L. elongata* var *L. playfairiana* var *minor*) and one species of *Phacus* (*P. mammillatus*) and four (*P. balatonicus* var *major*, *P. acuminatus* var *barrackporensis*, *P. caudatus* var *maj ranula* var *brevicaudatus*) are considered new. Two species and two varieties of *Lepo spirogyra* Korsh, *L. steinii* Lemm, *L. steinii* var *suecica* Lemm. and *L. ovum* var *ve* Prowse) and twelve species of *Phacus* (*P. lefevrei* Bourr, *P. nannos* Pochm, *P. wettste P. textus* Pochm, *P. carinatus* Pochm, *P. formosus* Pochm, *P. obolus* Pochm, *P. ranul P. sesquitortus* Pochm, *P. atrakoides* Pochm, *P. glaber* (Defl) Pochm. and *P. hispidul* Lemm. and one variety (*P. ranula* var *africana* Bourr) appear to be new records for t region.

Although 13 taxa of *Lepocinclis* (5 species and 8 varieties) and 79 taxa of *Phacus* (and 18 varieties) have been reported previously from the region there are only a fe which give full details. Some of the records also appear to be doubtful.

Keys to the taxa of the two genera described and lists of other taxa of the genera from the region are also given.

Latin diagnosis of *Euglena tuba* Carter *non* Johnson emend. Philipose 1982 is validate the species. Three additional species, *viz E. orientalis* Walton, *E. elastica* Pre *E. clara* Skuja are also described, the last being a new record for India.

Keywords. Periplast; striae; verrucae; pharyngeal cleft, paramylum; cyst; Indi Euglenineae.

1. Introduction

This account is the second in the series on Euglenineae, part one being on th *Euglena* Ehr (Philipose 1982).

and seven taxa of *Phacus* from Museum Pond, Madras. Gonzalves anc observed three taxa of *Lepocinclis* and one of *Phacus* at Bombay. Skuj accounts of 2 taxa of *Lepocinclis* and 15 taxa of *Phacus* from Burma, of wh latter was a new variety. Biswas (1949) stated that *Phacus pleuronectes* w organism found in the plankton of Indian inland waters.

In an account of the Euglenineae from Hyderabad, India, Suxena (19 two taxa of *Lepocinclis* and eighteen taxa of *Phacus* of which two varietie were new. Philipose (1960) reported that a number of species of *Lepocincl* commonly occur in Indian inland freshwaters. Naidu (1962, 1966) descril *Phacus* from Andhra Pradesh. Kamat (1961–62) recorded three taxa c (including a new variety) and 11 taxa of *Phacus* (including two new v Ahmedabad. Again, Kamat (1963, 1964, 1968, 1974, 1975) and Kama (1976) reported the occurrence of four taxa of *Lepocinclis* and thirty *Phacus* (including a new species and a new variety) from Maharashtra species reported by Kamat and Freitas (1976) is *L. texta*. Kamat (1967) one species of *Phacus* from Rajasthan and five species of the same genus fr Pradesh (Kamat 1968a). Singh (1948) reported *Phacus hameli* All *et* Le Pradesh and Hirano (1966) two species of *Phacus* from Kabul, Hortobàgyi (1969) described two taxa of *Lepocinclis* and six taxa of *Phac* reservoirs on the river Jamuna, Uttar Pradesh. Kachroo (1960), and Sing (1969) recorded *Phacus pleuronectes* in West Bengal and Uttar Predesh and Zafar (1959) and Munawar (1972) in Andhra Pradesh. Seenayya (1 two species of *Phacus* from the same State. Bharati and Hosmani (1973) and Bharati (1975) recorded *P. caudatus* Huebner and *Lepocinclis ovun* from Karnataka. Hosmani (1976) also described a new species of *Phacus* f State. Suxena *et al* (1973) described four taxa of *Lepocinclis* and six taxa of Kerala. Venkateswarlu (1976, 1981) reported one species of *Lepocinc* species of *Phacus* from Andhra Pradesh. Subba Raju and Suxena (19 *Lepocinclis ovum* and *Phacus indicus* Skv from the Himalayas. Patel and (1981) described 23 taxa of *Phacus* from Gujarat. Ashtekar (1982) reporte of *Lepocinclis* and 15 species and 4 varieties of *Phacus* from Aurangabad (1982) 4 species of *Phacus* from Rajasthan.

Altogether 13 taxa of *Lepocinclis* covering 5 species and 8 varieties, a *Phacus* comprising 61 species and 18 varieties have so far been recor Indian region. In a study of Euglenineae of north-east, central and south

therefore, been considered necessary to give detailed descriptions and figures
taxa reported here.

2. Locations and dates of collection

The collection centres are the same as given in Part I (Philipose 1982). A few ad
locations not included therein are given below:

2.1a *Assam*: (4a) Joyasagar N P 23 (30-5-55); (5a) N P 16 (6-4-55); (17a) N P,
(25-10-59); (18a) SDO's tank, Nazira (15-6-55).

2.1b *W. Bengal*: (26a) Tank D, Belgharia (27-6-50); (27a) Sagore Dutt
Kamarhati (30-5-50); (33a) Mukherjee's Pond, Hooghly (22-11-49); (36a) A
bund, Chandrakona Road (17-12-52).

2.1c *Bihar*: (37a) River Dehri at Hurka Nala (23-5-53).

2.1d *Madhya Pradesh*: (40a) Duatia Pond, Goharganj (20-7-54); (43a) Raj
Raipur (21-4-56).

2.1e *Orissa*: (45a) Treasury tank, Balasore (20-12-52); (55a) Municipa
Mayurbhanj (21-12-52); (77a) Ananda Sagar Pond, Narasingpur (17
(84a) Pond, Athmalik (14-4-56); (90a) B. Sagar, Sonepur (17-12-54); (93a
Subba Island, Kausalyagang (10-4-51); (100a) Public tank, Jeypore (19-2-57)

2.1f *Andhra Pradesh*: (103a) Dyke's Tank, Visakhapatnam (4-12-54); (104a)
tank, Narasingpatna (5-12-54); (111a) Gajjalu Tank, Ellore (7-12-54);
Vellapadia Tank, Kakinada (5-12-54); (112b) Cheedila Tank, Kakinada (6-1

2.1g *Karnataka*: (130a) K R Sagar drain pool, Mysore (8-2-53); (131a)
Nandi Hills (6-2-53); (133a) Chandracharis Pond, Coorg (9-2-53).

2.1h *Kerala*: (137a) Canal with *Lemna*, Manjaliparamp, Azhicode (2
(142a) Mankuzhikulam, Azhicode (26-2-49).

striae, very rarely smooth; with a single flagellum which is usually longer t
vacuolar system as in *Euglena*; eye-spot near the reservoir; chromatophor
parietal, discoid or irregularly polygonal and without pyrenoids; param
two large lateral ring-like refractory bodies, rarely small spherical or rod
median or in the posterior half. Mostly in freshwater.

Key to the taxa described

I. Periplast with striae

(A) Striae longitudinal

(a) Cell elongate-spindle shaped with hind end drawn out into a short end capitate; paramylum 2, ring- or shell-like; cell 35–39–64 × 7·9 .... 1.

(b) Cell fusiform to ellipsoid with apex truncate; paramylum usually

With short tail having no basal bulge; cell 22–30 × 7·5–17 μm.....

Tail with a basal bulge; cell 20–30 × 9·5–15 μm ....

(B) Striae spiral

(a) Striae from left to right

Anterior orifice slightly towards one side; cell ovoid with numerou rod-like paramylum; cell 36–60 × 26–45 μm ....

(b) Striae from right to left

Anterior orifice apical; paramylum usually 2 rings, rarely otherwis

(i) Cell ellipsoid to ovoid and usually with a short tail × 13–23·5 μm; tail up to 7 μm ....

Cell usually ellipsoid; tail with a basal bulge; cell 30–42 × 17–

....

Cell regularly ellipsoid; tail very small and acuminate or knob-li × 5–15 μm .... var

Cell ovoid and with a small conical tail; striae finely punctat × 20–21 μm; tail up to 3·7 μm long.................. var pu

Cell ovoid; paramylum disc-like and more than 2; cell 21–23

....

Cell ellipsoid to ovoid with a sharp pointed tail; membrane w verrucae; cell 33–35 × 15–17 μm .... va

(II) Periplast smooth and without striae
Anterior orifice not apical
Cell broadly ellipsoid with a well-developed slightly oblique tail; anteri
beaked; paramylum 2 rings; cell 35·6–40–50 × 17–28 μm; tail 10–16 × 4 μ
....9. *L. playf*
Cell and tail smaller; cell (with tail) 28–30 × 13·7–14·7 μm; tail 3·7–4·5 μ
....var *minor* v

1. *Lepocinclis marssonii* Lemm. emend. Conrad 1935 (figures 1a–b)

Conrad 1935, pp. 14–15, figure 4; Huber-Pestalozzi 1955, p. 140, figu
= *L. marssonii* Lemm. 1905; 1910, p. 508, figure 20 (p. 483); Allorge *et* Lefèvı
p. 130, figures 77–78.

Cell elongate-fusiform with the anterior end drawn out into a beak and the pc end gradually tapering to a tail; membrane hyaline, colourless and longitu striated; paramylum two, lateral and ring- to shell-like; cell 35–63·5 × 7·9–11·3 alone 5·3–9·4 μm.

Habitat: Stray in locations 26a and 30; common in location 136a.

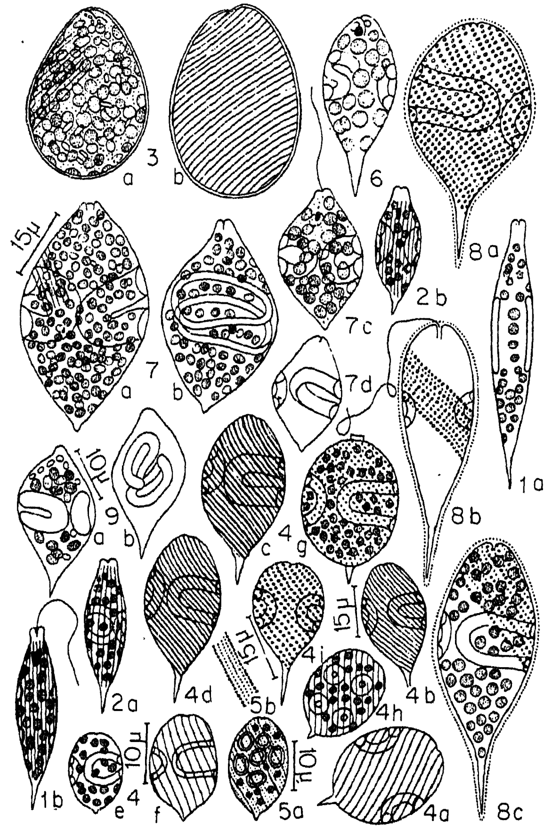
The organism agreed well with the typical species except that lower limits of were less, and it was narrower than the dimensions given by Conrad × 10–17 μm) and Lemmermann (39–40 × 11–13 μm). The tail was also more c than in Conrad's figures. In this respect it showed a certain resemblance to *L. m* var *elongata* Bourrelly (1961, pp. 202–203, plate 4, figures 6–7) with dimens 45–53 × 8–9 μm, but the tail did not arise from a basal bulge as in Bourrelly's

Distribution in Indian region: Maharashtra (Kamat 1975); W. Bengal and Ke

2. *Lepocinclis steinii* Lemm. emend, Conrad 1935 (figure 2a)

Conrad 1935, p. 17, figure 7; = *L. steinii* Lemm. 1904; 1913, p. 134, figure

Cell fusiform to ellipsoid; anterior end nearly entire or drawn out into a beak very end indented or flattened; with a short conical tail; membrane usually coloured and with longitudinal parallel striae which are uniform or alterna thicker; chromatophores numerous, small and polygonal to round or paramylum 1-3 ringlets or small ovoid granules; eye-spot fairly large;



Figures 1–9. **1a–b.** *Lepocinclis marssonii* Lemm. emend. Conrad; **2a.**
emend. Conrad; **2b.** *L. steinii* var *suecica* Lemm; **3a–b.** *L. salina* Fritsch;
Lemm; **4b–d.** *L. ovum* var *buetschlii* (Lemm) Conrad; **4e–f.** *L. ovum* var *di*
4g. *L. ovum* var *punctato-striata* Lemm; **4h.** *L. ovum* var *discifera* Conra
verrucosum Prowse; **5a–b.** *L. lefevrei* var *cuttackensis* var *nov* (**5b.** diagr
striae); **6.** *L. elongata* var *minor* var *nov*; **7a–b.** *L. fusiformis* (Carter) Lemn
7c–d. *L. fusiformis* fa *lemmermanni* Conrad; **8a–c.** *L. spirogyra* Korsh.; **9a**
var *minor* var *nov*. [Same magnification: (1a–b; 2a–b; 3a–b; 4a
7a–d; 8a–c); (4b); (4f); (4i); (4h; 5a); (9a–c)].

hick and with spiral striae running from left to right; chromatophores numerous an iscoid; paramylum also numerous, small and granular to rod-like and distributed i he cytoplasm; eye-spot a fairly large disc at the anterior end; flagellum not observed ell 30–45 × 23–30·8 μm; striae about 7 in 10 μm.

Iabitat: Abundant in location 127, common in locations 5a, 6, 29 (January) and 131 tray to rare in locations 17, 33 (March), 37, 43a, 50, 63 (N P 24, 25, 27, 29 August Jovember), 81, 85, 88a, 104, 105, 114–116, 118, 132, 133a and 157.

The organism agreed fairly well with the typical species but was slightly smalle 36–60 × 26–45 μm, Conrad), but was larger than var *minor* (Huber-Pest.) Conra 34–37·5 × 27–28 μm 1935, p. 60). Unlike in var *minor* the emargination at the anterio nd was regular as in the typical species. Hence it is kept under the typical specie Iortobágyi (1969) gives dimensions of 41·6–45 × 31·2–34 μm for his UP material.

It is not clear on what basis Kamat (1961–62, p. 20, figure 3) created a new variety, *vi* ar *ahmedabadinensis* since it does not differ in any way from the typical species. Th limensions given (50–55 × 35–38 μm) fall within the limits of the typical species.

The organism resembles *L. texta* but differs chiefly in that the spiral striae in th atter run from right to left.

Distribution in Indian region: Gujarat (Kamat 1961–62 as *L. salina* var *ahmedabadiner is*); Maharashtra (Kamat 1963, also as the above variety); Uttar Pradesh (Hortobág 969); Assam, W. Bengal, Bihar, Madhya Pradesh, Orissa, Andhra Pradesh, Karnatak nd Tamilnadu (!).

I. *Lepocinclis ovum* (Ehr) Lemm. 1901 (figure 4a)

Lemmermann 1910, pp. 504–05, figure 13 (p. 483); Conrad 1935, pp. 33–3 figure 25; as fa *typica* Huber-Pestalozzi 1955, p. 149.

Cell usually broadly ellipsoid to ovoid with pharyngeal opening exactly apica osterior end continuing as a small tail of variable length; pellicle with spiral stria which are either uniform, or moderately or markedly thicker ones alternating wit hinner ones; chromatophores small, numerous and discoid; paramylum two later ings; cells 20–38 × 13–23·5 μm; tail up to 7 μm.

Habitat: Stray to very rare in locations 5, 5a, 29, 45a, 57, 73, 76, 93, 103a, 118 and 13

Kamat (1963, 1964) gives dimensions of 25–38 × 13–18 μm, and Suxena *et al* (197 36–37 × 27–28 μm. As stated by Conrad (1935), the shape of cell, nature of striae an

1949, p. 163; Gonzalves and Joshi 1946, p. 175, plate 5, figure 10; *non L. bu*
Lemm. in Drezepolski 1925, figure 139.

Cell elongate-ellipsoid; pellicle usually yellowish brown in colour with unifo
moderately to markedly thickened striae alternating with thinner ones; tail
characteristic basal swelling; cell 38·7–42·2 × 18·5–20·3 μm; tail alone 7–10·6 μ

Habitat: Stray to very rare in locations 61 and 136a.

Conrad (1935) gives dimensions of 30–42 × 17–24 μm, Skuja (1949) 40–44 (inc
tail) × 20–22 μm, and 32–34 μm without tail, Prowse (1958) 50 (without tail) ×
tail alone 15 μm, and Hortobágyi (1969) 45·5 × 21·5 μm.

Distribution in Indian region: Maharashtra (Gonzalves and Joshi 1946); Uttar P
(Hortobágyi 1969); Burma (Skuja 1949); Orissa and Kerala (!).

var *dimidio-minor* Defl. 1924 (figures 4 e–f)

Deflandre 1924, p. 1124, figures 25–28; Conrad 1935, pp. 42–43, figure 33.

Differs from the typical species in the cell being ellipsoid to subcylindrical with a s
tail and the cell (17–19·5 × 11–14 μm) being much smaller.

Habitat: Stray in locations 23, 29 (December), 73 and 151 (November).

The variety agreed well with Deflandre's organism which measured
× 5–15 μm.

Distribution in Indian region: Gujarat (Kamat 1961–62, p. 20); Maharashtra (
1968, p. 98); Kerala (Suxena *et al* 1973, p. 334); W. Bengal, Orissa and Tamilna

var *punctato-striata* Lemm. 1905 (figure 4g)

Lemmermann 1910, p. 505, figure 14 (p. 483); Conrad 1935, p. 45, fig
Skvortzov 1937, p. 71, plate 9, figure 5.

Differs from the typical species in the presence of a tube-like neck in the cell th
which the flagellum emerges; striae punctate; cell 28–35 × 22–23 μm with the tai

var *discifera* Conrad 1935 (figure 4h)

Conrad 1935, p. 47, figure 39.

Differs from the typical species in the paramylum being in the form of perfora and not in rings, and their number being more than two (usually 4–6); cell 21 × with tail 1·8 μm; tail in the form of a teat.

Habitat: Stray in location 92.

The organism from Kausalyagang (location 92) had slightly narrower ce slightly longer tail, Conrad's specimens measuring 21–23 × 16–17·5 μm.

Distribution in Indian region: Maharashtra (Kamat and Freitas 1976); Oriss

var *verrucosum* Prowse 1958 (figure 4i)

Prowse 1958, pp. 154–55, figure 2s.

Cell oblong-ovoid with a sharp pointed tail; anterior end truncate-rounded with spiral rows of fine verrucae running to the left; cell 33 × 15 μm with t 10 μm.

Habitat: Stray in locations 134 and 136a.

It agreed well with Prowse's variety measuring 35 × 17 μm.

Distribution in Indian region: Kerala and Karnataka (!).

5. *Lepocinclis lefevrei* Conrad 1935

Conrad 1935, pp. 64–65, figure 60.

Cell narrow-ovoid with the hind end slightly broader and with a stout obtuse like tail; pellicle with spiral striae which are beaded; alternate striae sometin stronger beading; paramylum as two lateral rings; chromatophores numerou and discoid; cell 21–30 × 12–17 μm. Known only from Belgium.

var *cuttackensis* var *nov*. (figures 5a–b)

the number of paramylum and shape of cell, the paramylum are in rings an
perforated discs, the striae are beaded and the cell is narrower.

Distribution in Indian region: Orissa (!).

6. *Lepocinclis elongata* (Swirenko) Conrad 1935

Conrad 1935, p. 64, figure 59; = *L. fusiformis* var *elongata* Swirenko 1928.

Cell fusiform with the anterior cleft slightly towards one side; posterior end prolo
into a conical tail; pellicle with spiral striae; paramylum as two rings; cell 5
× 23 μm. This species is known only from Russia.

var *minor* var *nov*. (figure 6)

Cellulae minores quam forma typica; tantummodo 32·5 μm longitudine (cum ca
atque 17·5 μm latitudine, cauda 10 μm; chromatophora satis magna, disco
numerosaque; stigma prope extremitatem anteriorem; striae non observatae.

Habitatio: Aberrans in loco Sagore Dutt Pond-2, Kamarhati, W. Bengal dicto
27a) 30-5-1950.

var *minor* var *nov*. (figure 6)

Cell smaller than in the typical species, (32·5 μm in length, including tail; 17·5 μ
breadth, with tail 10 μm); chromatophores fairly large, discoid and numerous; eye-
near anterior end; striae not observed.

Habitat: Stray in location 27a, 30-5-1950.

Distribution in Indian region: W. Bengal (!).

7. *Lepocinclis fusiformis* (Carter) Lemm. emend. Conrad 1935 (figures 7a–b)

Conrad 1935, pp. 49–51, figures 42–44; Prowse 1962, p. 111, plate 1, figu

emmermann's figure. Conrad (1935, figure 44) states that the organism sometim ccurs in a palmelloid condition with copious mucilage, when the periplast is usua iin.

istribution in Indian region: Maharashtra (Carter 1859); Burma (Skvortzov 193 erala (Suxena *et al* 1973); Assam, W. Bengal, Orissa and Kerala (!).

ı *lemmermannii* Conrad 1935 (figures 7c–d)

Conrad 1935, p. 52, figure 45; = *L. globosa* Francé var *fusiformis* Lemm. 1901; 19 p. 508

ell smaller than in the typical species and somewhat rhomboidal; flagellum nea ody length; eye-spot at anterior end; cell 25·5–30 × 13·8–15·8–18·9 μm.

abitat: Stray to rare in locations 23, 29 (December), 63 (N P 31, March), 1 February) and 152a.

The dimensions were slightly larger than those given by Conrad (14–21 × 8–11 μı he Indian organism actually comes midway between the typical species and *mmermannii*, but nearer the latter.

istribution in Indian region: W. Bengal, Orissa and Tamilnadu (!).

. *Lepocinclis spirogyra* Korshikov 1942 (figures 8a–c)

Korshikov 1942, pp. 25–26, figure 3.

ell fusiform with variable thickness and enclosed within a mucilaginous envelo nterior end broadly rounded; posterior end produced into a distinct spine; peripl ith spiral rows of brown, more or less rectangular, verrucae running from right to l nd making one turn round the body; beading with uniformly thick verrucae ternate rows showing weak beading; chromatophores discoid and numero aramylum two large median lateral rings; flagllum when observed about 2/3 bo ngth; cell 55–61 × 17·5–30 μm, with tail alone 10·5–14·5 μm.

abitat: Rare to common in locations 136 and 136a; stray in locations 63, 73, 76 a 2.

9. *Lepocinclis playfairiana* (Defl) Defl 1932

Conrad 1935, pp. 67–68, figure 65; Hortobágyi 1943, p. 87, figure
fusiformis var *caudata* Playfair 1921, p. 127, figures 3 j-k; =*Crumenul*
Defl 1929.

Cell more or less fusiform with a slight depression on one side at the
thereby forming a beak at the tip; posterior end continuing as a fairly lon
usually bent slightly towards one side; chromatophores numerous
paramylum two large rings, with additional small rods or granules; per
and without striae; cell (including tail) 35·6–40–50 × 17–28 μm; tail
Known only from Australia, France and Hungary.

var *minor* var *nov*. (figures 9a–b)

Varietatas a varietate typica differens ut minor, cellulis tantummode 28
cauda) × 13·7–14·7 μm; cauda sola 3·7–4·5 μm.

Habitatio: Rara in stagno, in loco Chetput Fish Farm, Madras dicto (l
1943 et Vellore (loc. 159) 19-12-1954.

Differs from the typical species in its smaller size, the cell measuring o
(including tail) × 13·7–14·7 μm, with tail alone 3·7–4·5 μm.

Habitat: Rare in locations 154 (27 April 1943) and 159, (19 Decembe

Distribution in Indian region: Tamilnadu (!).

Other taxa of *Lepocinclis* reported from the Indian region are:

L. acuta Prescott, from Maharashtra (Ashtekar 1932)

L. glabra Drez. *f. minor* Prescott, from Maharashtra (Kamat 1975)

L. marssonii Lemm. emend. Conrad (1935) var *khannae* (Skvortzov
(1955) from Burma (as *L. khannae* Skvortzov 1937)

L. ovata (Playf) Conrad var *deflandriana* Conrad (1935) from Kerala
1973)

L. ovum (Ehr) Lemm var *indica* (Skvortzov) Huber-Pest. (1955). As *L. in*
from Burma (Skvortzov 1937) and from Andhra Pradesh (Suxena 1

L. ovum var *angustata* (Defl) Conrad (1935)—as *L. ovum* var *angusta*

system as in *Euglena*. A single flagellum which is usually longer than the body. E
near the reservoir. Chromatophores numerous, parietal and usually dis
irregularly polygonal and without pyrenoids. Paramylum numerous and of d
shapes and size. Nucleus usually central. Mostly in freshwater.

Key to the taxa described

Subgenus Chlorophacus Pochmann 1942 (with chromatophores and green in

I. Cell mostly flattened like a lens or in optical cross-section three-edged; fre
with basal, lateral or dorsal thickening; pellicular striae longitudinal o
paramylum central or lateral, discoid, ring-like or watch-glass like, rarely r
cells 12–200 μm in size................ Section Proterophacus Pochma

(A) Cell without a tail; hind end entire or nearly so; round to elongate in out
in optical cross-section.

(a) Cell nearly round

(i) With a disc-like paramylum; cell (40–) 46–48 (–55) × 39–41·6 (–4
....1. *P*

(ii) With a pseudo-ring like paramylum; cell margin irregularly u
35·5–37 × 30·5–33·3 μm............................2. *P. bal*

Cell larger, 48·4 × 41 μm ....var *major*

(iii) With a number of medium-sized paramylum; number of paramyl
striae variable; cell 48–58 × 40–48 μm....................3. *P.*

(b) Cell more or less oval

(i) Breadth of cell about 3/5 of length; paramylum single; cell 14–14·
× (7·5–) 8 (–9) μm ....4. *P.*

(ii) Cell nearly two times as long as broad

(1) Paramylum usually two and disc-like; cell 17–18 (–19·8) × 7·9–8
....5. *P. we*

(2) Paramylum usually one and ring-like; cell slightly broader towa
end, 18–20 (–22) × (6–)7–9 (–11) μm......................6. *P.*

(B) Posterior end of cell with a blunt knob; pellicular striae spiral; par
lateral, large and shell-like; cell 12·5–17 (–17·6) × 8–13 μm........7.

(C) Cell with hind end cuneiform (wedge-like) and sometimes with a short
or slightly oblique tail; usually 3–5 edged

(a) Cell symmetrical

Paramylum 1–2 small rings or several discs; cell (18–)22–30 × (13–)17–2

(ii) Striae nearly vertical on one side and spiral on the other sid
× 22–23.5 µm ....

(b) Cell in two diagonal halves and usually longer than broad;
oblique to curved tail; cell 23–27 (–40) × (16·8–) 26–27 (–30)
....13.

(F) Cell bent, twisted or asymmetrically inflated

(a) Cell bent at an angle; dorsal side with a keel, ventral side wi
paramylum usually two; cell (27–)35 (–45) × (8–) 10–12 (–19) µm
....14. *I*

(b) Cell strongly twisted

(i) With one twist; cell triangular in outline with a perfora
paramylum at each angle; tail short and like a teat; cell 35–37
..................................15. *P. mammil*

(ii) In two twisted halves separated by two furrows; paramylum
irregular discs; cell (34–) 40–48 × 24–32 µm ....16.

(c) Cell with two unequal asymmetrical halves with one lobe shorter
longer lobe with a tail and one large central paramylum;
× 22–23 µm; tail 4–6 µm.............................1

Inflation less than in type; cell larger with closer striae; tail
paramylum 2–3 medium-sized discs and additional smaller one
× (28–)30–33 µm; tail 7·5–9·4 µm........................var

(G) Cell with well developed, straight, oblique or curved tail

(a) Tail oblique, paramylum one or two; cell margins entire

(i) Paramylum two central concentric ones of unequal size; cell
or slightly longer than broad and slightly asymmetrical; cell
× (20–) 30–50 µm; tail 5–10 µm .... 18. *P.*

(ii) Paramylum a single large central disc; cell broadly oval; (43–)4
30–32 µm; tail 9–15 µm 19

(b) Tail curved; paramylum two discs; cell margins entire

(i) Cell narrowed at both ends, elongated and slightly asymmetric
like; cell (25–) 37–55 × 20–35 µm 20

(ii) Cell nearly round or slightly longer than broad, 3-edged in c
section; with a large central and a smaller excentric paramylu
50–100 × (28–) 30–60 µm ....21. *I*

(+ +) Anterior paramylum very large and nearly filling cell; cel
× 20 (–22·5) μm . . . . 24A. *P. fo*

(3) Flanks of cell entire; cell nearly rectangular; paramylum a lar
besides smaller ones; tail straight; cell 28–33 × 13–20 μm 24B. *P.*

(ii) Paramylum a single central ring; cell flat with flanks irregularly cre
undulate; cell without a dorsal keel; tail slightly bent; cell 50–80 × 30
. . . . 25. *P. un*

(iii) Paramylum usually a large central disc; one or both margins with
constriction; tail curved; cell (including tail) 30–42 × 22–35 μm. 25A.

(H) Cell with a medium-sized wedge-shaped tail.
Paramylum ring-like or ring- and disc-like; Cell about 2½ times as long a
with tail ¼–⅓ body length; cell 86–105 (–130) × 25–50 (–55) μm; tai
23–30 μm . . . . 26. *P*

(I) Cell long and thin with a tail as long as the body or shorter or longer.

(a) Cell usually flat or slightly twisted at anterior end only

(i) Tail usually irregularly twisted or bent at end, about half body lengt
an angle to hind end of cell; paramylum 3, rarely more; cell (exclud
up to 80 μm, 37–55 μm broad, tail 37–55 μm . . . . 27. *P*

(1) Tail shorter and nearly straight; paramylum more than two discs
cell (excluding tail) 74–83 × 38·4–47·5 μm, tail 24·5–33 μm . . . . .
. . . . var *brevicaudatus*

(2) Tail recurved; with 2 large central paramylum appearing like a b
lens and additional discoid ones; cell (40·5–) 47–50 × (27·3–) 33
tail 33–35 μm . . . . var

(ii) Tail not twisted and about body length; paramylum usually one, ra
concentric discs.

(1) Cell heart-shaped, lateral margins usually entire, paramylum ri
cell (74–) 85–190 × (27·5–) 40–70 (–75) μm . . . . 28. *P. lon*

(+) Cell elongate-elliptic, 100–150–170 × 35–50 μm; paramylum
or ring-like . . . . var.

(+ +) Cell oval, (146–) 170–188 × 40–65 μm; tail up to 100 μm
mylum ring-like . . . . va

(+ + +) Cell club-shaped or with hind end narrower and asymn
110–120 × 41–44 μm; paramylum a central disc . . . . var *at*

(2) Lateral margins of cell with a notch; paramylum ring-like; cell

(2) Cell with $1\frac{1}{2}$ twists and only one ridge; paramylum r
(excluding tail) 62–90 × 40–54 μm. . . . . 31A. *P*

(3) Cell twisted like a snail shell with a dorsal and ventral wing;
central disc; cell 70–120 × 30–54 μm. . . . . 32.

II. Cell pear- or spindle-shaped and thick, sometimes triangular or slig
pellicle composed by thick ridges; paramylum one or two and late
ventral; chromatophores usually numerous, small and discoid, ra
lateral . . . . Section Pleurapsis Poc

Cell with many small chromatophores, ribs running towards left and w

(a) Tail shorter than body, straight or slightly curved; cell pear-shape
longer than broad, 30–55 × 15–21 μm . . . .

(b) Tail a prolongation of the body; cell spindle-shaped, much longe
and 3-edged in optical cross-section, 28–30 × 9–10 μm . . . . 34.

III. Cell usually flattened like a lens; pellicle smooth or with longitudina
are punctate or with crests; paramylum two lateral pads . . . .
. . . . Section Acanthopeltis Poc

(A) Cell wall smooth or with rows of fine punctae; cell 26–27 × 14·5-
. . . .

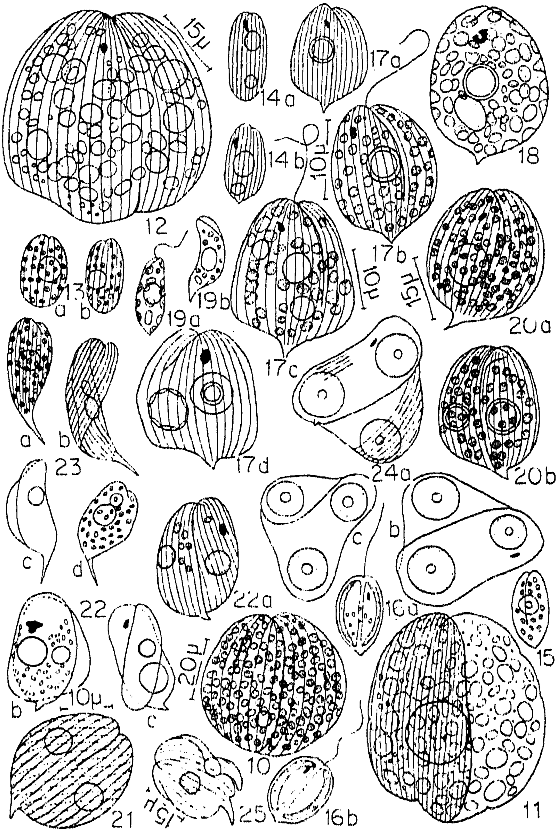
(B) Cell wall with cone-like warts
Warts arranged in longitudinal rows
Cell longer than broad, breadth 2/3–3/5 times length, 25–36 × 1
6–11 μm thick. 3

(C) Pellicle with longitudinal rows of small spines.
Spines pointed towards the apex; cell 30–40–55 × 18–33 μm. . . 37.

Sections Kampyloter Pochmann 1942 and Dolichoplastes Huber-Pe
with one species each, and Subgenus *Hyalophacus* Pochmann (1942) (with
with one species, not reported from the Indian region so far.

Section I. Proterophacus Pochmann 1942

1. *Phacus stokesi* Lemm. 1910 (figure 10)



Figures 10–25. **10.** *Phacus stokesi* Lemm.; **11.** *P. balatonicus* var *major* var shown only in one half of cell); **12.** *P. lefevrei* Bourr.; **13a–b.** *P. nannos* Poch *P. wettsteinii* Drez.; **15.** *P. pusillus* Lemm.; **16a–b.** *P. agilis* Skuja; **17a–c.** *P.* (Stokes) Huber-Pest.; **17d.** *P. acuminatus* var *barrackporensis* var *nov*; **18.** *P. bra* Pochm. (striae not shown); **19a–b.** *P. inflexus* (Kissel.) Pochm.; **20a–b.** *P. curvic* **21.** *P. textus* Pochm.; **22a–c.** *P. anomalus* Fritsch *et* Rich; **23a–d.** *P. raciborskii* D *P. mammillatus* sp. *nov* (three views of same individual); **25.** *P. contortus* Bo magnification: (10); (11; 12; 13a–b; 14a–b; 16a–b; 17a, d; 18; 19a–b; 22a–c; 23a–d; 24 (17c); (20); (21); (25)].

2. *Phacus balatonicus* Hortobágyi 1943

Hortobágyi 1943, pp. 87–88, figures 27–36; Huber-Pestalozzi 1955, p
figure 194A.

Cell broadly oval to egg-shaped and sometimes with irregularly undulate sides
ends broadly rounded; apical furrow reaching the hind end; periplast with fairly
longitudinal striae; chromatophores numerous and disc-shaped; paramylum i
form of a "pseudo-ring" (thick in the periphery and thin towards the middle) ne
apex or towards the side of the cell; additional watch-glass like paramylum also pr
cell 35·5–37 × 30·5–33 μm.

Reported so far only from Hungary.

var *major* var *nov* (figure 11)

Varietas a varietate typica et a var boglariense Hortob. (Hortobágyi 1943, fi
40–42) differens ut paululo maior; grana paramyli aliquantulum unum latus vers
propius extremitatem posteriorem sita; cellula 48·4 × 41 μm.

Habitatio: Aberrans in loco Thandankulam, Azhicode, Trichur dicto (loc. 136a)
1949.

Differs from the typical species and var *boglariensis* Hortob. (36.7–39 × 27–29.
(Hortobágyi 1943, figures 40–42) in its slightly larger size; paramylum slightly to
one side; nearer the hind end; cell 48·4 × 41 μm.

Habitat: Stray in location 136a.
Distribution in Indian region: Kerala (!).

3. *Phacus lefevrei* Bourrelly in Bourrelly *et* Manguin 1952 (figures 12, 12a)

Huber-Pestalozzi 1955, pp. 182–83, figure 196B; Prowse 1958, p. 160, figur
Bourrelly 1961, p. 305, Plate 4, figure 13.

Cell broadly ellipsoid with both ends rounded; posterior end broader with a fairly
swelling; anterior end with a small depression; furrow extending from front to hin

measured 49 × 43 μm, had 15 striae and 25 paramylum mostly of medium siz
another specimen from location 26a the cell measured 48·5 × 47·5 μm, there we
thirty paramylum of various sizes and twenty-one striae. The latter had also a sn
spot. However, the Indian specimens did not differ materially from Bourrelly's
and is, therefore, retained under it.

This species resembles *Phacus segreti* Allorge *et* Lefèvre (see Pochman
figure 4) in general shape, but the latter differs in having only two large para
discs and the striae are sometimes slightly spiral.

Distribution in Indian region: Assam, W. Bengal and Orissa (!).

4. *Phacus nannos* Pochmann 1942 (figures 13a–b)

Pochmann 1942, p. 123, figure 5.

Cell elongate with broadly rounded ends and without any acute point at the hi
pellicular striae longitudinal; chromatophores small, numerous and discoi
mylum one large circular disc; cell 15·7 × 7·5–9 μm.

Habitat: Stray in location 23.

The organism agreed fairly well with Pochmann's species (14 – 14·7 × 8
nannos differs from *P. wettsteinii* Drez. (see below) in its smaller size and the h
being always rounded.

Distribution in Indian region: W. Bengal (!).

5. *Phacus wettsteinii* Drezepolski 1925 (figures 14a–b)

Drezepolski 1925, p. 267, Tafel 3, figure 122; Pochmann 1942, pp. 123–24, f

Cell elongate-oval with both ends rounded or with a sharp point at the hind enc
groove reaching up to the hind end; pellicle hyaline and with longitudina
paramylum usually two rounded discs; cell 17·8 – 19·8 × 7·9 – 8·5 μm.

Habitat: Stray in location 29 (January).

The organism agreed fairly well with the European and Malaysian (see Prow
figure 3a) ones but the paramylum was sometimes slightly elongate as in the Ma

striae spiral to nearly longitudinal; paramylum 1–2 and ring-like; cell
18·5 × 8 μm.

Habitat: Stray in locations 28 (April), 29 (April), 33 (April, July and Septembe
and 154.

Distribution in Indian region: Andhra Pradesh (Suxena 1955; Naidu 1966);
Pradesh (Hortobàgyi 1969); Maharashtra (Ashtekar 1982); W. Bengal, Karnata
Tamilnadu (!).

7. *Phacus agilis* Skuja 1926 (figures 16a–b)

Conrad 1938, p. 7, figures 18–22; Pochmann 1942, pp. 134–35, figure 21; H
Pestalozzi 1955, p. 189, figure 215.

Cell-shaped like a coffee bean and elongate-ellipsoid to nearly four-cornered; an
end slightly narrowed; posterior end also narrowed and obtuse with a knob-like
line with the longitudinal axis; pellicle with fine slightly spiral striae; flagellum
body length; paramylum two, lateral and like the shell of a mussel
15·8 – 17·6 × 9·7 – 11·4 μm.

Habitat: Stray in location 21 (November), rare in location 33 (July).

Distribution in Indian region: Andhra Pradesh (Seenayya 1972); Gujarat (Pate
Waghodekar 1981); W. Bengal (!).

8. *Phacus acuminatus* (Stokes) Huber-Pest. 1955 (figures 17a–c)

Huber-Pestalozzi 1955, p. 192, figure 224; = *P. acuminata* Stokes
Lemmermann 1913, pp. 138–39, figure 233; Skuja 1949, p. 163; = *P. acumi*
subsp. *americana* Pochmann 1942, p. 141, figures 32 a–c.

Cell broadly oval to triangular or nearly spherical with the anterior end sli
narrowed and rounded; posterior end broader and ending in a short sharp poi
conical tail; apical groove usually reaching up to the hind end; pellicular s
longitudinal; chromatophores small, numerous and discoid; paramylum one to

var *barrackporensis* var *nov*. (figure 17d)

Cellula late ovalis et apululum asymmetrica, plana et 4–5 angulata, sulco apica
usque ad circa tertiam partem longitudinis corporis; striae pelliculates prom
grana paramyli tria, duo anuliformia inaequaliaque, tertium discoideum; stig
magnum; extremitatas posterior caudam brevem obtusamque quae angulum
centra axem longitudinem format habens; cellula 31·5 × 27 μm, cauda sola

Habitatio: Aberrans in cisterna stationis pro investigatione, Barrackpore (
30-11-1949.

Cell broadly oval and slightly asymmetrical, flat and 4–5 angled, with th
groove only up to about one-third the body length; pellicular striae pro
paramylum three, two ring-like and unequal, the third disc-like; eye-spot fair
posterior end with a short obtuse tail which is at a slight angle to the longitudi
cell 31·5 × 27 μm with tail alone about 3 μm.

Habitat: Stray in location 29 (November).

Though the organism resembles *P. acuminatus* var *indica* (Pochm) Huber-Pe
(1955, figure 229) in its shape, the tail is shorter and not so sharply pointed, th
furrow does not extend to the posterior end and the number and nature of par
are different. It resembles *P. acuminatus* var *acuticauda* (Roll) Huber-Pest
figure 230) somewhat in its general shape, short nearly straight tail, two par
rings of unequal size and its dimensions, but differs in having a third
paramylum. So, it is treated here as a new variety.

Distribution in Indian region: W. Bengal (!).

9. *Phacus brachykentron* Pochmann 1942 (figures 18, 18a)

Pochmann 1942, p. 145, figure 33; Suxena 1955, pp. 437–38, figures 6–7

Body more or less elliptical to oval and slightly asymmetrical with a short str
slightly bent tail; paramylum 2–3; when three in number, two central and co
and the third excentric to the central ones; when two, one slightly lateral and the
excentric to the first; eye-spot usually small and disc-like, but sometimes ho
shaped; cell 21·5–35 × 15–26 μm.

Habitat: Stray in locations 29 (November) and 154.

from a dorsal hump; pellicular striae delicate and somewhat spiral; par one large and the other small, ovoid to discoid or elongate and rod-lik usually on the dorsal side; chromatophores discoid and numerous; ey flagellum about body length; cell 13·5–19·4 × 6·3–7 μm.

Habitat: Stray in locations 23, 30 and 33 (April).

The organism from W. Bengal is slightly smaller than Pochmann's s measures 24 × 7–10 μm. Patel and Waghodekar (1981) give dimensio 27·2 × 5·3–11 (– 13.2) μm for their specimens from Gujarat.

Distribution in Indian region: Gujarat (Patel and Waghodekar 1981); W

11. ***Phacus curvicauda*** **Swirenko 1915 (figures 20a–c)**

Pochmann 1942, pp. 155–58, figures 49–51; Suxena 1955, p. 439, fi Prowse 1958, p. 167, figures 3f, 3k; Hortobágyi 1969, p. 32, plate 5,

Cell nearly round with the anterior end slightly narrow and the hind broadened and bearing at its tip a short tail which is turned slightly towa apical groove of variable length ranging from about 1/3 to nearly the full cell; pellicular striae longitudinal; chromatophores numerous and discoid usually two discs or rings of unequal size lying laterally, very rarely only discoid; cell (including tail) 20–25–30 × 16·7–20–27·5 μm; tail 2·5–5 μ

Habitat: Stray in locations 28 (May), 29 (February–March), 30, 33 (May 151 (February, October).

Pochmann gives dimensions of 20–35 × 18–25 μm, with 27 × 22– typical, Skuja (1949) 35 × 28 μm, Suxena (1955) 20–30·5 × 22–25·5 μm, P 23–28 × 22–25 μm, Kamat (1963, 1964) 30–36 × 22–32 μm and Waghodekar (1981) 21·3–37·7 × 16–29·3 μm. Hortobágyi (1969) gives d 28–29·5 × 20–23·4 μm.

Distribution in Indian region: Burma (Skuja 1949); Andhra Pradesh (S Maharashtra (Kamat 1963, 1964, 1975, Kamat and Freitas 1976; Ash Gujarat (Patel and Waghodekar 1981); Himachal Pradesh (Kamat 1

Habitat: Stray in location 33a.

The organism was slightly smaller than the one given by Pochmann which
26–30 × 23–25·5 μm.

Distribution in Indian region: W. Bengal (!).

13. *Phacus anomalus* Fritsch *et* Rich 1929 (figures 22a–d)

Fritsch and Rich 1929, p. 78, figures 24 H–N; Pochmann 1942, p. 163, fig 60.

Cell in two unequal halves with one of the halves in the form of a wing; cell lor broad or nearly as broad as long and winged in apical view; ends broadly roun the hind end somewhat broadened and with a short bent tail; pellicul longitudinal to slightly spiral; paramylum usually two, disc-like and unequa one in each half of the cell, rarely three, two in one half of the cell and the thi other half; one of the paramylum sometimes against the side of the cell and in an hour glass; cell 23·5–28·2 × 17·6–24·6 μm, with tail 1·8–2·1 μm.

Habitat: Stray to rare in locations 2 (June), 23, 28 (April–May), 29 (Janu (May), 30, 40, 61 (N P 9, 10 and 16-May), 82, 89 and 91a.

Fritsch and Rich (1929) give dimensions of 23–27 (without tail) × 26–27 thickness of 17–22 μm while Prowse (1958) gives them as 30–40 × 25–30 μm and Waghodekar (1981) as 23·6–33 × 16·8–24·2 μm. It is possible that Pr mixed up *P. anomalus* and *P. inflatus* var *pterophorus* Skuja (see elsewhe account) since his figure 3x for *P. anomalus* looks very much like that of Skuja though slightly smaller with only one paramylum.

Distribution in Indian region: W. Bengal (Kachroo 1960); Maharashtra (Ka Freitas 1976; Ashtekar 1982); Gujarat (Patel and Waghodekar 1981); As Bengal, Madhya Pradesh and Orissa (!).

14. *Phacus raciborskii* Drezepolski 1925 (figures 23a–e)

Drezepolski 1925, p. 266, Tafel 3, figure 113; Pochmann 1942, pp. 174–7 73–74; Bourrelly 1961, p. 305, plate 4, figure 15.

15. *Phacus mammillatus* sp. *nov.* (figures 24a–c)

Cellula torta, uno latere velut ala replicato; a superficie visa triangularis, rotundatis, unum discum paramyli perforatum satis magnum, magnitudin in omni angulo visum; cauda parva, mammiformis ad extremitatem poster aliformes; striae pelliculares obscurissima longitudinales ad spirales, et tor sequunter; stigma lineiforme, ad extremitatem alae anteriorem; cellula 35 31·7 μm, *ca.* 14 μm, crass.

Habitatio: Rarissima in N P 13 in Jobra, Cuttack (loc. 61) 2-8-1952 et Joyasagar (loc. 9) 16-3-1966.

Iconotypus: Figures **24a–c**.

Cell twisted with one side folded back as a wing; triangular in surfac broadly rounded angles and with one fairly large perforated paramylum d the same size in each corner; with a small papilla-like tail at the posterio wing-like fold; pellicle with very faint striae which are longitudinal to spira the twist of the cell; eye-spot streak-like and at the anterior end of the win 37 × 30–31·7 μm and about 14 μm thick.

Habitat: Very rare in locations 9 (March) and 61 (N P 13-August).
Type: Figures **24a–c**.

The organism comes near *P. anomalus* Fritsch *et* Rich in its being in tw differs in having three perforated paramylum discs of more or less equal tail being very small and teat-like. So, it is treated here as a new specie

Distribution in Indian region: Assam and Orissa (!).

16. *Phacus contortus* Bourrelly in Bourrelly *et* Manguin 1952 (figure

Huber-Pestalozzi 1955, pp. 204–5, figure 260A; Rino 1972, p. 152, pla 17–20.

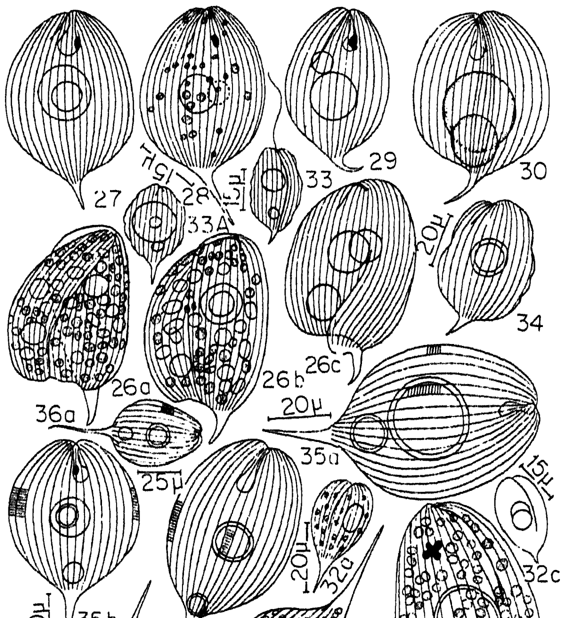
Cell oval and in two asymmetrical twisted halves separated by two broad f one half spread out in the form of a wing which is projected backwards like a stout oblique tail from the hind end of the other half; periplast with faint l striae following the twist; paramylum two regular or irregular discs, one in

Cell in two unequal, inflated, asymmetrical lobes with one lobe longer and t
expanded more in the transverse direction; longer lobe with a short tail; paran
large ring in the long lobe; pellicle with longitudinal striae; eye-spot marked; fl
long; cell 25–32 × 22–23 μm; tail 4–6 μm. The typical species is known on
Australia.

var *pterophorus* Skuja 1949 (figures 26a–c)

Skuja 1949, p. 164, Tafel 36, figures 29–32.

Inflation of cell less than in the typical species; cell larger with closer
chromatophores small, discoid and numerous; paramylum two to three discs o
the same size or one slightly larger; sometimes a smaller one superimposed on th



one and looking ring-like; with additional paramylum bodies of smaller size; straight or slightly curved at the tip to one side; cell 49–54·5 × 28–32 μm with 7·5–9·4 μm.

Habitat: Rare in location 23 and stray in locations 1, 39, 61 (N P 12- May), 105.

The species agreed well with Skuja's variety measuring 47–55 × 30–3 thickness 20–22 μm, but the tail in the Indian material was sometimes more curved towards the tip.

Distribution in Indian region: Burma (Skuja 1949); Assam, W. Bengal, Pradesh, Orissa and Andhra Pradesh (!).

18. *Phacus pleuronectes* (OFM) Duj 1841 (figure 27)

Lemmermann 1910, p. 512, figure 4 (p. 483); Pochmann 1942, pp. 180–8 82–84; Suxena 1955, p. 440, figure 22; Hortobàgyi 1969, p. 32, plate V,

Cell broadly oval and asymmetrical due to slight twisting, with one side mo than the other; apical groove reaching up to nearly the middle of the cell; pos with a sharp tail bent at an angle towards one side; pellicular striae longitu running to part of the tail due to torsion of the cell; paramylum two, one lar other small, the latter superimposed on the former; body of cell 40–45 × 2 with tail up to 7 μm.

Habitat: One of the commonest species observed in many locations. Co location 73, rare in locations 38 and 93a; stray in locations 2, 20, 29, 33, 36a, 5 25, 29 – August, November), 70, 76, 89, 90a, 91, 93, 105, 115, 118, 124, 132, 137, 145, 151 (August, March), 157 and 159.

Lemmermann (1910, 1913) gives measurements of 45–49 × 30–33 μm, P (1942) 40–80 × 30–50 μm, while Suxena (1955) gives them as 31–37·5 × 20 Kamat (1961–62) 37–50 × 37–63 μm and Hortobágyi (1969) 34–38 × 26–2 author's specimens came between Lemmermann's and Hortobágyi's. Su (1973) give dimensions of 105–110 (including tail) × 40–45 μm for their C material which is exceptional. Maximum breadth of 63 μm given by Kamat (is also quite high.

Cell broadly oval and flat, slightly asymmetrical and with a sharply poi
tail; paramylum a single large or medium-sized central disc; apical fur
reaching up to the middle only; cell (with tail) 48–54 × 29–32 μm; tail alon

Habitat: Stray in locations 33 (April, June), 151 (August), 154 and 15
Drezepolski (1925) gives the dimensions as 50–56 × 32–32·5 μm, w
15 μm, Pochmann (1942) as 46–52 μm in length, and Prowse (195
tail) × 30 μm, tail alone 9 μm.

Distribution in Indian region: Maharashtra (Kamat 1975; Kamat and F
Ashtekar 1982); W. Bengal and Tamilnadu (!).

20. *Phacus hamatus* Pochmann 1942 (figure 29)

Pochmann 1942, pp. 182–84, figure 86; Prowse 1958, p. 169, figu
pleuronectes var *citriformis* Drezepolski 1921–22; 1925, p. 266, Tafel 3
Skvortzov 1937, p. 74, Tafel 10, figure 3.

Cell elongate-oval and somewhat asymmetrical with both ends narrowed a
at middle or slightly towards the base; lips at front end equal or uneven
frequently larger; tail bent characteristically like a hook with its base
paramylum usually two discs of unequal size, super-imposed and appearin
sometimes lying apart; apical furrow reaching up to the middle or slightly
spot fairly large and nearly triangular; cell (without tail) 35–38 × 23–
6–9 μm.

Habitat: Stray in locations 90 and 151 (March).
Drezepolski and Pochmann give its dimensions as 38–45–55 × 25–35
7·5–10 μm, whereas Prowse gives them as 37–45 × 20–25 μm with tail

Distribution in Indian region: Burma (Skvortzov 1937); Gujarat (Kama
Orissa and Tamilnadu (!).

21. *Phacus orbicularis* Huebner 1886 (figure 30)

Lemmermann 1913, p. 138, figure 256; Pochmann 1942, p. 178, fig

with tail 14 μm looks more like a *P. triqueter* (Ehr) Duj (see Pochmann 19
since the paramylum are shown as two separate rings of more or less equa
in *P. orbicularis* they are two overlapping, rarely concentric, discs of
Suxena's organism from Hyderabad measuring 35–46·5 × 28–31·5 μm
al's (1973) one from Kerala measuring 44–45 × 37–38 μm were smalle
the dimensions of 50–100 × 30–60 μm given by Pochmann. Skuja
dimensions of 100–102 × 48–50 μm for his *P. cingeri* from Burma.

Distribution in Indian region: Maharashtra (Gonzalves and Joshi 1946,
13; Kamat 1964, 1968, 1975; Ashtekar 1982); Andhra Pradesh (
Himachal Pradesh (Kamat 1968a); Kerala (Suxena *et al* 1973); Burma
P. cingeri Roll); Assam, W. Bengal, Orissa, Karnataka and Kerala (

22. *Phacus carinatus* Pochmann 1942 (figure 31)

Pochmann 1942, pp. 187–88, figure 91; –*P. triqueter* (Ehr) Duj in
p. 124, plate V, figures 8–11.

Cell resembling that of *P. pleuronectes* in shape but differing in bein
and angular with a sharply pointed oblique to curved tail which follow
the cell; pellicular striae longitudinal; paramylum two discs of un
concentrically in one half of the cell or lying apart, one in each hal
(including tail) 34 × 24 μm; tail 5–5·5 μm.

Habitat: Stray in location 134.
Distribution in Indian region: Karnataka (!).

23. *Phacus caudatus* Huebner 1886 (figures 32a–c)

Lemmermann 1913, p. 138, figure 237; Pochmann 1942, pp. 146–
Suxena 1955, p. 438, figure 3; Hortobágyi 1969, p. 31, plate V, fi
var *ovalis* Drez 1925, p. 266, figure 111; Conrad 1938, p. 8, figur
minor Drez 1925, p. 266, figure 107.

Cell oval, slightly twisted and asymmetrical with a dorsal keel reachi
... produced into a more or less straight tail w

Kamat's (1964), Bombay material measured 43–47 × 20–22 μm and his Ko
(Kamat 1963) 46–50 × 28–31 μm; Organisms from Gujarat (Kamat 196
and Waghodekar 1981) measured 40–50 × 23–28 μm and 21·4–42·5 × 10
respectively. Hortobágyi's (1969) organism measured 30–33 × 15–17
author's collections, the smaller form measuring 25–28 × 12·5–14·5 μm
only from one locality, viz 28, and one individual (see figure 32c) from
measuring 32 × 17·5 μm resembled var *ovalis* in which the paramylum o
overlapping discs of equal size.

Naidu's (1966, figure 23) *P. trifacialis* Prowse from Andhra Pradesh
31 × 15 μm could be a *P. caudatus* since there are two paramylum rings of u
in his organism whereas Prowse's (1958, p. 166) species measuring 22-
13 μm has only a small central ring and the cell is 3-angled in optical cr

Distribution in Indian region: Andhra Pradesh (Suxena 1955); Maharash
1963, 1964, 1968, 1975); Gujarat (Kamat 1961–62; Patel and Gaghodekar 1
Pradesh (Hortobágyi 1969); Karnataka (Bharati and Hosmani 1973);
Orissa and Karnataka (!).

var *major* var *nov*. (figure 32d)

Cellula plus minusve plana ellipsoideaque ad oblongam, extremitate
paululo latiore, et in caudam brevem, rectam, crassam, cuneïformem
sulcus apicalis solum usque ad circa tertiam partem longitudinis cell
paramyli ut duos anulos inaequales, maiore prae, minore post nucleum
(?), omnibus tribus in linea in axe longitudinali cellulae ordinatis; stigma cr
extremitatem anteriorem; cellula multo maior quam in specie typica
cauda) × 35 μm, cauda sola 9·2 μm.

Habitatio: Aberrans in cisterna stationis pro investigatione, Barrackpo
11-4-1950.

Cell flat and ellipsoid to oblong with the posterior end slightly broader an
a short, straight, wedge-shaped, stout tail; apical groove up to about a t
length of the cell only; paramylum two unequal rings with the larger one i

24. *Phacus ankylonoton* Pochmann 1942 (figure 33)

Pochmann 1942, pp. 148–49, figure 37.

Cell elongate-oval with a dorsal thickening; more or less three-edged in o
section; flanks of cell entire or irregularly crenate; paramylum two,
longitudinal axis, with the anterior one larger; tail straight or slightly curve
striae longitudinal; cell $37 \times 18\ \mu m$.

Habitat: Stray in location 28 (April).

Distribution in Indian region: Gujarat (Patel and Waghodekar 19
W. Bengal (!).

24A. *Phacus formosus* Pochmann 1942 (figures 33A, $33A_1$)

Pochmann 1942, p. 149, figure 38.

Cell elongate-oval with the lips at the anterior end somewhat curved and a
usually straight: flanks of cell entire or slightly and irregularly crenate; in o
section three-edged; with a dorsal thickening from the anterior to the ve
end; paramylum two, the anterior one being disc-like or ring-like and e
large nearly filling the cell; the posterior one small, slightly elongate and d
lying very near the larger one or partly overlapped by it; eye-spot not obser
$41 \times 22{\cdot}5\ \mu m$; tail alone $6{\cdot}5\ \mu m$.

Habitat: Stray in locations 18a and 73.

The organism agreed well with Pochmann's species except that it
broader, the breadth given by Pochmann being about $20\ \mu m$. The large e
could not be observed in the preserved material.

Distribution in Indian region: Assam and Orissa (!).

24B. *Phacus obolus* Pochmann 1942 (figure 33B)

Pochmann 1942, p. 153, figure 43; $=$*P. caudatus* var *lata* Allorge *et* L

Cell more or less rectangular and about twice as long as broad with the

Cell oval and asymmetrical with the anterior half narrower; tail small ar
lateral margins of cell irregularly crenate; pellicular striae longitudinal; p
one large central ring or two unequal discs arranged concentrically or
overlapping; cell 55 × 30–35 μm.

Habitat: Stray in locations 28 (May), 73 and 104a.

Pochmann (1942) gives dimensions of 50–80 × 30–48 μm.

Distribution in Indian region: Maharashtra (Kamat and Freitas 1976); V
Orissa and Andhra Pradesh (!); Burma (Skuja 1949).

25A. *Phacus onyx* Pochmann 1942 (figure 34A)

Pochmann 1942, pp. 192–93, figures 98 a–d, Suxena 1955, p. 440, figures
Prowse 1958, p. 171, figures 3 m–n, x.

Cell slightly asymmetrical and more or less trapezoidal with the posterior
and with a sharply curved tail; anterior end narrower; one or both margins
a notch; pellicle with longitudinal striae; paramylum a single large disc (
rarely two smaller ones; cell (including tail) 40–47 × 28–32·5 μm; tail a
10 μm.

Habitat: Stray in locations 28 (April), 63 (N P 31-March) and 73 (Febru

Pochmann gives its dimensions as 30–42 × 22–35 μm, Suxena as 3
26·5 μm and Prowse as 50–55 × 35–37 μm with tail 14–17 μm. In Suxena
there were two notches on one margin and none on the other.

The organism differs from *P. undulatus* in that the lateral margins are n
the tail is more sharply curved and in its usually smaller size. The paramy
more commonly a single central disc.

Distribution in Indian region: Andhra Pradesh (Suxena 1955); Maharasht
1963; Kamat and Freitas 1976; Ashtekar 1982); W. Bengal and Orissa (!).

26. *Phacus meson* Pochmann 1942 (figures 35a–d)

Pochmann 1942, pp. 195–96, figure 102; Suxena 1955, pp. 440–42, figu

76, 77a, 83, 84, 86, 88, 89, 93, 103a, 104, 115, 133a, 136a and 152a (F

The species agreed well with Skvortzov's (1937) organism from
differed in the presence of transverse striae between the longitudina
majority of specimens and the lateral margins of cells in all the specim
being entire, none with lateral notches being seen. Apart from the cells
larger than Skvortzov's (89–93 × 25–40 μm), occasionally three paramy
observed. Suxena's (1955) organism from Hyderabad (100–102 × 49-
transverse striae agreed with the author's but he, like Prowse (1962, plate
the Malaysian specimens (75–80 × 50 μm; tail alone 30 μm) observ
paramylum. No transverse striae were observed by Prowse. Kamat (1
breadth of his Alibag specimens as up to 55 μm.

Naidu (1966, p. 28, figure 24) gives dimensions of his *P. meson* from
200–220 × 85–90 μm. He does not give further details except mentio
larger than the typical *P. meson*. His figure showing two paramylum ri
that of *P. meson*. If its dimensions are correct, it will have to be treated as
viz P. meson var *major* (Naidu) var *nov*. since it is much larger than the t

Kamat's (1964, figure 1) *P. maharastrensis* from Bombay with cylindric
having dimensions of 110–130 × 45–52 μm, with tail 40–45 μm, app
slightly larger *P. meson*. In fact it resembles Skvortzov's (1937) Burm
measuring 93 × 25 μm in all respects (see Pochmann 1942, figure 103
Pestalozzi 1955, figure 294c), except size, the fewer striae (eight compared
in Skvortzov's figure) and the slightly larger paramylum. The striae are a
correctly in Kamat's figure.

Distribution in Indian region: Burma (Skvortzov 1937), Andhra Pra
1955); Maharashtra (Kamat 1968); Assam, W. Bengal, Madhya Pra
Andhra Pradesh, Karnataka, Kerala and Tamilnadu (!).

27. *Phacus ranula* Pochmann 1942 (figures 36a, g, h)

Pochmann 1942, p. 212, figure 126; Huber-Pestalozzi 1955, p. 227, fig
only; Prowse 1958, p. 172, figures 4 g, h.

Cell more or less elliptic to oval and flat or slightly twisted; anterior

var *brevicaudatus* var *nov.* (figures 36b–d)

Including *P. ranula* Pochmann *p.p.* in Huber-Pestalozzi 1955, p. 227, f
only.

Varietas a varietate typica differens ut cauda brevior et fere recta aut cacu paulum flexa, non, autem torta, cellula necnon crassior quam varietas typica laterales cellulae aut integri aut interdum incisi; striae transversae i longitudinales praesentes absentesve; grana paramyli quattuor ad num coidea, annularia aut ambo, magnitudine variantia; stigma distinctum; fla observatum; cellula (cauda exclusa) 74–83 × 38·5–47·5 μm, cauda 24·5–3

Habitatio: Rara in palude, Kausalyagang, Puri (loc. 92) 10-4-1951 et a loco Thandankulam, Azhicode, Trichur dicto (loc. 136a) 26-2-1949.

Differs from the typical species in the tail being shorter and nearly strai very slightly bent towards the tip, but not twisted, also stouter than in the t margins of cell either entire or sometimes with a notch; transverse striae absent; paramylum four to numerous, ring-like, disc-like or both and of va eye-spot distinct; flagellum not observed; cell (excluding tail) 74–83 × 38· tail 24·5–33 μm.

Habitat: Rare in location 92 and stray in location 136a.

Huber-Pestalozzi (1955) states that though the tail of *P. ranula* is usua irregularly it is occasionally straight. His figure 313e also shows that the ta than in the typical species. There are also numerous paramylum bodies. S author's specimens with numerous paramylum were exactly like Huber-I specimens. Since the material from both the locations mentioned consistently short nearly straight tails, it appears to the author that it coul as a new variety, *viz* var *brevicaudatus*. Though the organism looks very m *meson* it can be distinguished from it by the origin of the tail at an angle to th end of the cell and the larger number of paramylum.

Distribution in Indian region: Orissa and Kerala (!).

var *africana* Bourrelly 1961 (figures 36e–f)

Bourrelly 1961, p. 305, figures 7–8.

28. Phacus longicauda (Ehr) Duj 1841 (figures 37a–b)

Lemmermann 1910, p. 511, figure 24 (p. 483); Huber-Pestalozzi 1955, 299; = *Euglena longicauda* Ehr 1838; = *Phacus longicauda* su Pochmann 1942, pp. 199–200, figures 109–10.

Cell usually obovate or cordate, sometimes otherwise, asymmetric flattened and broadest in the front one-third or half; anterior end broadly with uneven lips; tapering towards the posterior end evenly and more or l end in a long straight or distinctly curved tail; pellicle with longitudinal converge towards the ends; lateral margins of cell usually entire, rarely paramylum one to two (concentric or lying apart) discs; cell (includi 141 × 45–47·5 μm; tail 59–64 μm.

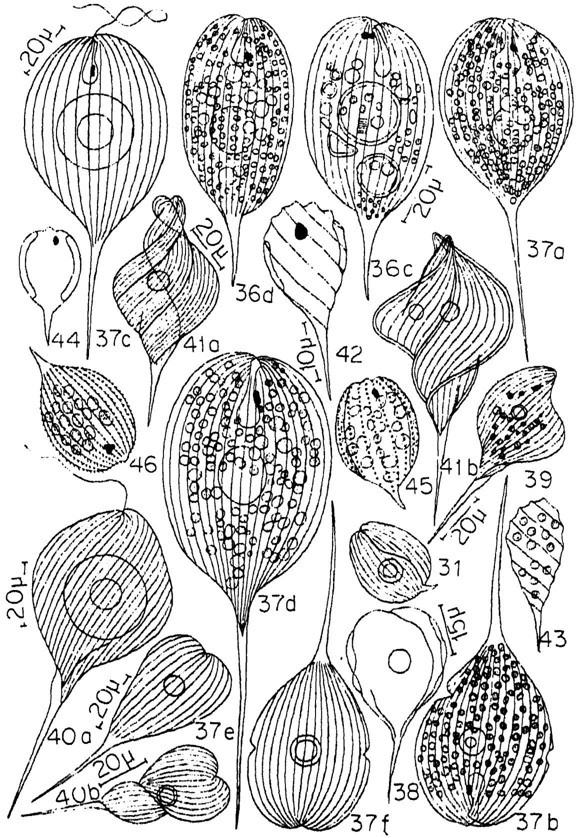
Habitat: Stray to very rare in locations 4, 17, 23, 33 (October), 38, 76, 78, 127 and 151; rather common in location 93a.

Measurements given by Pochmann (1942) are 85–190 (including tail) and by Skuja (1949) 80–130 × 31–57 μm. According to Huber-Pest Pochmann's *P. longicauda* subsp. *cordata* has to remain as the typical spe original species described by Ehrenberg was cordate, and Pochmann has typical species. In *P. longicauda* the eye-spot is described as large, but in material it was small though distinct. Though the paramylum in the auth was usually in the form of a ring, occasionally there were 2–3 separate disc ring.

Kamat (1961–62, 1963, 1964) gives dimensions of 80–110 × 40–7 specimens from Gujarat and Maharashtra while Suxena *et al* (1973) give 114 × 85–86 μm with tail 95–96 μm for their Kerala material.

Distribution in Indian region: Kashmir (Bhaṭia 1930); Gujarat (Kam Maharashtra (Kamat 1964, 1968, 1973, 1974; Kamat and Freitas 1 (Suxena *et al* 1973); Burma (Skuja 1949); Assam, W. Bengal, Bihar, O Pradesh, Karnataka and Tamilnadu (!).

var *rotunda* (Pochmann) Huber-Pest. 1955 (figures 37c, g, h)



Figures 31, 36–46. 31. *P. carinatus* Pochm., **36c–d.** *P. ranula* var *brevi*
nov. **37a–b.** *P. longicauda* (Ehr) Duj; **37c.** *P. longicauda* var *rotunda* (Pochm.)
37d. *P. longicauda* var *maior* Swir.; **37e.** *P. longicauda* var *attenuata* (Pochm.) Hu
P. longicauda var *insecta* Kocz.; **38.** *P. ephippion* Pochm.; **39.** *P. cicumflexus* Poc
tortus (Lemm.) Skvortz.; **41a–b.** *P. helikoides* Pochm.; **42.** *P. pyrum* (Ehr)
atrakoides Pochm.; **44.** *P. glaber* (Defl) Pochm.; **45.** *P. suecicus* Lemm.; **46.** *P. hisp*
Lemm. [Same magnification: (31, 37e); (36c–d; 37a–b, d; 41a); (37c; 39); (38); (40
45, 46); (42)].

35–140 (including tail) × 35–50 μm, with tail alone 40–60 μm. Obvi

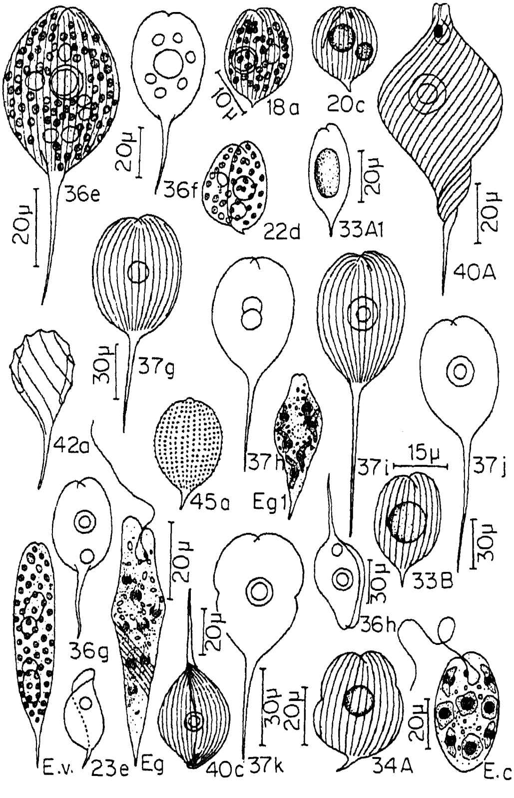


Figure 18a. *P. brachykentron* Pochm.; **20c.** *P. curvicauda* Swir.; **22d.** *P. ar*
Rich; **23e.** *P. raciborskii* Drez.; **33A1.** *P. formosus* Pochm., side view; **33B.** *I*
34A. *P. onyx* Pochm.; **36e–f.** *P. ranula* var *africana* Bourr.; **36g–h.** *P. ranul*
P. longicauda var *rotunda* (Pochm.) Huber-Pest.; **37i–j.** *P. longicauda* var
P. longicauda var *insecta* Kocz.; **40A.** *P. sesquitortus* Pochm.; **40c.** *P. tor*
42a. *P. pyrum* (Ehr) Stein; **45a.** *P. suecicus* (Lemm) Lemm.; **Ec.** *Euglena clar*
E. granulata (Klebs) Schmitz; **Ev.**? Free living cell of *E. vaginicola*
magnification: (18a); (20c, 22d, 23e, 36e, 42a, Ev); (33A1, 33B); (36g–h);
Eg1); (37g–h); (37i–j); (37k); (40A); (40c)].

var *attenuata* (Pochm) Huber-Pest. 1955 (figure 37e)

Huber-Pestalozzi 1955, p. 222, figure 301; = *P. longicauda* subsp. Pochmann 1942, p. 202, figures 112 a–c; = *P. longicauda* (Ehr) Duj in 1937, p. 73, Tafel 10, figure 2.

Cell flat and elongate-oval to club-shaped with the anterior end broader; po narrow and asymmetrical; tail about body length; with a central disc-like p cell 83 × 30 μm; tail alone 35 μm.

Habitat: Stray in locations 40, 76 and 98a.

Distribution in Indian region: Burma (Skvortzov 1937); Madhya Pra Orissa (!).

var *insecta* Koczwara 1915 (figures 37f, k)

Huber-Pestalozzi 1955, p. 223, figure 303; Skvortzov 1937, p. 73, Tafel = *P. longicauda* subsp. *insecta* (Koczwara) Pochmann 1942, p. 204, fig

Cell pear-shaped with the tail more or less bent, and with a notch on e margin of the cell; paramylum single, central and ring-like; cell (incl 90–120 × 42–45 μm; tail alone up to 60μm.

Habitat: Stray in locations 5, 17, 45, 61 (N P 11–May), 63 (N P 38–Septemb 84, 88 and 127.

Pochmann and Huber-Pestalozzi give dimensions of 80–130 × 35– Skvortzov 136 × 47 μm.

Distribution in Indian region: Burma (Skvortzov 1937); Assam, Orissa and (!).

29. *Phacus ephippion* Pochmann 1942 (figure 38)

Pochmann 1942, p. 208, figure 120; Suxena 1955, p. 442, figures 32 *longicauda* var *torta* Lemm. in Fritsch and Rich 1929, p. 76, figures B–

Cell pear-shaped and curved like a saddle with folds along the margins; ar

Cell more or less asymmetrical and more unequally bent than twistec
somewhat pointed with the lips close together; posterior end with a st
slightly twisted tail; pellicular striae prominent and spiral; apical furrow
the middle; paramylum central and ring- or spool-shaped or oval, som
additional basal grain; cell 73–90 × 34–45 μm.

Habitat: Stray in locations 33 (April, May, November), 63 (N P 24–Au
in location 151 (January).

Pochmann gives dimensions of 79–90 × 34–45 μm for this species.

Distribution in Indian region: Burma (Skvortzov 1937); Andhra Pr
1955); Maharashtra (Kamat 1975); W. Bengal, Orissa, Andhra
Tamilnadu (!).

31. *Phacus tortus* (Lemm) Skvortzov 1928 (figures 40a–c)

Pochmann 1942, p. 209, figures 121 a–d; Suxena 1955, p. 443, figure
1969, p. 32, plate 5, figure 60; = *P. longicauda* var *torta* Lemm. 191C
1949, p. 164.

Cell slightly twisted, the number of twists being usually one, and usually
relation to the middle line; anterior and posterior ends narrowed, broa
posterior end produced into a long, straight or slightly bent tail
longitudinal striae which follow the twist; with one large or medium
ring-like (or two super-imposed disc-like) paramylum; flagellum abou
cell (including tail) 79–82·5 × 31·5–34 μm; tail alone 30·5–33 μm.

Habitat: Widely distributed. Stray in locations 4, 20, 28 (May), 29 (Ju
33 (April), 40a, 57, 61 (N P 16–May), 76, 83, 88, 89, 90, 90a, 93, 101

Pochmann (1955) gives measurements of 80–112 (including tail), m
80–90 × 38–52 μm, 40–45 μm being the more common breadth. Su
dimensions as 73–79 × 34–38 μm and Prowse (1958) 80–85 (inc
27–36 μm, with tail alone 30–33 μm, while Kamat (1963, 1964) gives 7C
40 μm for his Maharashtra material. Hortobágyi's (1969) organism
70·2 × 24·7–30·5 μm, with tail 18–23 μm. Naidu's (1966, figure 25) *P. t*
lateral folds towards the anterior end and measuring 77–100 × 40–? 4

twists; with a moderately long tail; paramylum usually a central ring; with a large -like eye-spot at the anterior end; cell (including tail) 90·6 × 42·2 μm; tail alone μm.

itat: Stray in location 33 (April).

ochmann gives its dimensions as 62–90 × 40–54 μm and states that morphologi- y the organism comes between *P. tortus* and *P. helikoides*.

ribution in Indian region: W. Bengal (!).

Phacus helikoides **Pochmann 1942 (figures 41a–b)**

ochmann 1942, p. 212, figure 125; Prowse 1958, p. 172, figure 4f; = *P. torta* Lemm) var *tortuosa* Skvortzov 1928; 1937, p. 72, Tafel 9, figure 14.

spirally twisted (usually 3 twists) with the anterior end slightly narrowed and ing in a forked cleft; cell markedly broad in the anterior third, and with a straight line tapering tail at the posterior end; outline of cell like a delta; dorsal and ventral s forming a spiral keel from the middle of the cell and appearing more pronounced ards the posterior end which appears four-sided in optical cross-section; pellicular e spiral and following the twists; paramylum a central ring or disc, rarely two; cell luding tail) 91–111 × 42–49 μm, with tail alone 31–38 μm.

itat: Common in locations 33 (April) and 61 (N P 15–May); stray in locations 56, 90, 91, 126, 133a and 136a.

ochmann (1942) gives its measurements as 70–120 × 30–54 μm while Kamat 54) gives them as (67–) 80–110 × 32–40 μm. Malaysian specimens (see Prowse 8) measured 95–100 μm (including tail) × 42 μm, tail 25 μm.

ribution in Indian region: Burma (Skvortzov 1937); Maharashtra (Kamat 1964); Bengal, Orissa, Karnataka and Kerala (!).

tion II. *Pleurapsis* Pochmann 1942

Phacus pyrum **(Ehr) Stein 1878 (figure 42, 42a)**

Distribution in Indian region: Burma (Skuja 1949); Assam, W. Bengal, Oriss
Pradesh, Kerala and Tamilnadu (!).

34. *Phacus atrakoides* Pochmann 1942 (figure 43)

Pochmann 1942, p. 219, figure 133; = *P. pyrum* (Ehr) Stein in Skvortzov 1
Tafel 9, figure 22.

Cell spindle-shaped and in optical cross-section three-angled; anterior enc
posterior end gradually drawn out into a pointed tail; pellicle with spiral ri
to the left; chromatophores numerous and discoid; cell 28–30 × 9–10 μm

Habitat: Stray in location 112a.
Pochmann (1942) gives its dimensions as 30 × 10 μm.

Distribution in Indian region: Burma (Skvortzov 1937); Andhra Pradesh (

Section III. *Acanthopeltis* Pochmann 1942

35. *Phacus glaber* (Defl) Pochmann 1942 (figure 44)

Pochmann 1942, p. 236, figures 160 a–d; Prowse 1958, p. 173,
= *P. hispidula* (Eichw) Lemm. fa *glabra* Deflandre 1930.

Cell broadly oval, elliptic in side view and with an acicular or awl-shaped
posterior end; anterior end with a short papilla; cell wall smooth; paran
lateral pads; eye-spot prominent; chromatophores small, numerous and d
(including tail) 26·5 × 14·5 μm; tail alone 7 μm.

Habitat: Stray in location 67.
Pochmann does not give its dimensions but states that the species is esse
P. suecicus without wart-like excrescences. Prowse gives its dimensions as 2

Distribution in Indian region: Orissa (!).

36. *Phacus suecicus* (Lemm) Lemm 1913 (figures 45, 45a)

emmermann (1913) gives its measurements as 34 × 20–21 μm while Pochmann 2) gives them as 19–22 (mostly 20) μm, with thickness 6–11 μm. Playfair (1921) s them as 30–34 × 23–24 μm with thickness 6 μm and tail 7–8 μm in his *oniliata* var *suecica* Lemm.

ribution in Indian region: Burma (Skuja 1949); Maharashtra (Kamat 1963); Bengal and Orissa (!).

Phacus hispidula **(Eichw) Lemm 1910 (figure 46)**

emmermann 1910, p. 516; 1913, p. 139, figure 242; Pochmann 1942, p. 238, figure 52.

elongate-ellipsoid with the anterior end slightly retuse and with a short tubular ellar opening; posterior end with a short straight or sometimes slightly bent tail; cell e or less symmetrical; periplast with longitudinal rows of small forwardly pointed es; chromatophores small, numerous and discoid; paramylum two lateral pads; spot small to medium-sized; cell (including tail) 32–40·5 × 20–22·9 μm; tail alone -7·5 μm.

itat: Stray in locations 33 (July), 140, 140a and 142a.
emmermann (1913) gives its dimensions as 33–55 × 18–33 μm.

ribution in Indian region: W. Bengal and Kerala (!).
er taxa of *Phacus* reported from the Indian region are as follows:

. abruptus Korshikov 1928
from Gujarat (Patel and Waghodekar 1981).
. acuminatus (Stokes) Huber-Pestalozzi 1955
r *discifera* (Pochm) Huber-Pest. 1955–from Gujarat
(Kamat 1961–62); Maharashtra (Kamat 1975; Kamat and Freitas 1976);
r *granulata* (Roll) Huber-Pest. 1955
from Maharashtra (Ashtekar 1982).
r *indica* (Pochm) Huber-Pest. 1955
from Burma (As *P. triqueter* (Ehr) Duj-Skvortzov 1937, p. 74, Tafel 10, figure 4 only); Maharashtra (Kamat 1975);

4. *P. alatus* Kleos 1881–85
   Burma (Skuja 1949);
5. *P. anacoelus* Stokes 1885 (1888)
   Burma (Skuja 1949);
6. *P. angulatus* Pochmann 1942
   Burma (As *P. alata* var *indica* Skvortzov 1937 and *P. lemme*
   Skvortzov 1937 p. 74); Maharashtra (Kamat 1975; Ashtekar 198
7. *P. anomalus* Fritsch *et* Rich 1929
   var *pullus-gallinae* Nygaard 1949
   Maharashtra (Kamat and Freitas 1976; Ashtekar 1982). Patel and
   (1981) include this variety under their typical species;
8. *P. bharatii* Hosmani 1976
   Karnataka (Hosmani 1976);
9. *P. brevicaudatus* (Klebs) Lemm. 1910
   Burma (Skvortzov 1937); Maharashtra (Ashtekar 1982);
10. *P. caudatus* Huebner 1886
    var *tenuis* Swirenko 1915
    Gujarat (Kamat 1961–62);
11. *P. cylindraceus* Popova 1976
    Gujarat (Patel and Waghodekar 1981);
12. *P. cylindrus* Pochmann 1942
    Andhra Pradesh (Suxena 1955);
13. *P. dangeardi* Lemm. 1910
    Andhra Pradesh (Naidu 1966); Gujarat (Patel and Waghodekar
14. *P. fomini* Roll 1925 fa.
    Burma (Skuja 1949);
15. *P. granum* Drez 1925
    Gujarat (Patel and Waghodekar 1981); Maharashtra (Ashtekar
16. *P. hameli* Allorge *et* Lefèvre 1930
    Maharashtra (Kamat 1963, 1964; Ashtekar 1982); Gujara
    Waghodekar 1981);
17. *P. heimii* var *minor* Suxena 1955
    Andhra Pradesh (Suxena 1955). This variety reported by Naidu (1
    from Andhra Pradesh is doubtful since he shows in his figure only o

23. *P. minutus* (Playf) Pochmann 1942
Rajasthan (Kamat 1967); Maharashtra (Kamat 1975);
24. *P. musculus* Pochmann 1942
Gujarat (Patel and Waghodekar 1981);
25. *P. myersii* Skvortzov 1919
Gujarat (Patel and Waghodekar 1981);
26. *P. oscillans* Klebs 1881–1885
Burma (Skvortzov 1937); Andhra Pradesh (Seenayya 1972); Gujarat (
Waghodekar 1981);
27. *P. pekinensis* Skvortzov 1925
Burma (Skvortzov 1937); Maharashtra (Kamat 1964, 1975);
28. *P. peteloti* Lefèvre 1933
Gujarat (Patel and Waghodekar 1981);
29. *P. platalea* Drez var *minor* Kamat 1961–62
Gujarat (Kamat 1961–62); Maharashtra (Kamat and Freitas 1976;
1982);
30. *P. polytrophos* Pochmann 1942
Himachal Pradesh (Kamat 1968a); Gujarat (Patel and Waghodekar 1
31. *P. pseudonordstedtii* Pochmann 1942
Andhra Pradesh (Suxena 1955); Maharashtra (Kamat 1975);
32. *P. pseudoplatalea* var *indica* Kamat 1961–62
Gujarat (Kamat 1961–62);
33. *P. quinque-marginatus* Jahn *et* Shawh
Andhra Pradesh (Naidu 1966);
34. *P. skujae* Skvortzov 1928
Andhra Pradesh (Suxena 1955; Seenayya 1972); Gujarat (Patel and Wa
1981);
35. *P. spiralis* Allerge *et* Jahn 1943
Andhra Pradesh (Naidu 1966);
36. *P. stokesii* fa *minor* Conrad 1938
Maharashtra (Kamat 1975);
37. *P. strongylus* Pochmann 1942
Burma (as *P. setosa* var *crenata* Skvortzov 1928–Skvortzov 1937);
38. *P. swirenkoi* Skvortzov 1928
Maharashtra (Kamat 1975);

Naidu's *P. trifacialis* Prowse (see Naidu 1966, figure 23) could in all p
P. caudatus Huebner (see text under that species).

Addendum to Part I—Euglena (Philipose 1982)

Euglena tuba Carter 1869 *non* Johnson 1944 *emend.* Philipose 1982, pp. 5
20 a–r and aa–nn

Latin diagnosis:
Cellula manifeste metabolica, plerumque, autem, fusiformis ad ellipsoid amve, raro fere spherica, extremitate anteriore late rotundata, parte acumen obtusum aut caudem brevissimam terminanta, aut saepiu pellicula strias tenues spirales punctatas praebens; chromatophora c. 5 plerumque fusiformia aut manifeste curvata, raro sphaeroidea ad dis chromatophorum pyrenoideum bis vaginatum habens; grana paramyli a discoidea in cytoplasmate saepe visum; nucleus sphericus mediusque, fo satis parvum; flagellum *ca.* 1/3–1/2 longitudinis corporis; haematochr lucis clarae tetam cellulam, et tempore lucis minuentis extremitatem habentes; haematochroma rarissime nulla; cysta ampullae in fundo rot stipitem tubiformem in basim infundibuliformem terminatem habet co longitudine varians, saepe transverse striatus, et interdum irregularit basis ad oram pilis setiformibus interdum praedita; cystae intra matri aggregatae, stipitibus undique eminentibus, aut lateraliter in ordine lin inter se adhaerentibus; in statu encystate flagellum nullum; liberatio orga ampullae aut in parte media aut propius stipitem aut eius gelatinization liberationem flagellum breve apparet et longius gradatim factum; fla exutum; locomotio flagello praesente, libere natante effecta, aut sine lento formae mutationem comitata; cellula plerumque (31)–54–74– 45 μm aut longior, in cellulis fere sphericis, autem 41·5–56 × 36–43 μ 88 μm, cum ampulla 31–47 × (22·5–)-26–47 μm, stipis us que ad 70 μm (8·5–) 13–18 μm lat., basis 18–29–32 μm diam.

Habitatio: Ut spuma rubra vel viridis aut in placto generali aut in granulose satis frequens ad maxime abundantem in locis 7–9, 21, 24, 32, 66, 69, 72–75, 80, 104, 113, 116, 149 et 151 et in aliquot stagnis in locis

ı) whereas those observed in the same pond a few days later (23/26-9-1951) and suring 56–70·4 × 26·3–45 μm had spindle-shaped chromatophores (see figure). Both appeared to belong to the same taxon since the pyrenoid was always present ıe chromatophores (unlike in *E. orientalis* Walton described below). However, it not clear why individuals collected within such a short period as five days from the e pond should show different structure especially because there was no visible ıge in ecological conditions.

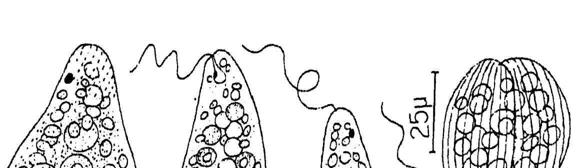
he association of cysts of *E. tuba* with those of *E. tuba* var *pseudotuba* f. *minima* ipose was also quite frequent in location 61.

hree other species of *Euglena* which could not be included in Philipose (1982) are ribed below. Additional points on a few specimens already described including or corrections are also given.

lena orientalis Walton 1915 (figures Eo1–Eo5)

ojdics 1953, p. 181; = *Euglena* sp. Kashyop 1908, pp. 111–112.

metabolic but usually cylindrical to ellipsoid and ending posteriorly in a short tail ail-like process rather abruptly; anterior end rounded; pellicle with faint spiral e which appear to be punctate; chromatophores green, spherical, of fairly uniform (about 6–8 μm in diameter) and without pyrenoids, distributed fairly uniformly le the cell or sometimes with one or both ends free of them; number of ›matophores about 8–21; paramylum numerous, discoid and usually smaller ximum length about 5·3 μm) than the chromatophores and more or less uniformly ·ibuted; eye-spot prominent and frequently towards one side at the anterior end rvoir small with the canal frequently opening slightly towards one side the cell ellum about 3/4 body length and easily discarded; nucleus not observed natochrome present or absent; when present, usually spread over the anterior two- ds of the cell and light brick red to deep orange in colour; cyst not observed, but a ıded cell with what appeared to be the broken remains of a cyst (see figure Eo4) was in one of the collections; cell 64–80 × 22–30 μm.



Habitat: As a brick red or green scum or in the general plankton of locatio
November 1953 and February 1954). During February 1954 it appeare
scum two days after heavy rains and the cell had no haematochrome
Eo2–Eo5)

The organism agreed fairly well with Kashyop's species measuring 62
125 × 31 μm, though Kashyop does not refer to the striae as punctate. He
the number of the chromatophores, but the largest paramylum is stated
7 μm long. The cyst is described as flask-like as in *E. tuba*. Since the
observed in the author's material the organism is referred to as *E. or*
tentatively. Kashyop did not observe pure green organisms. However, suc
were observed in the otherwise brick red *E. tuba* also by the author afte
(see Philipose 1982). Huber-Pestalozzi (1955) regarded this species
known and hence uncertain.

The organism differs from *E. tuba* with discoid chromatophores in th
pyrenoids in the chromatophores and in the presence always of a short ta
process.

Hortobágyi's (1960, figures 1–10) olive green *E. tuba* with a very short
spiral striae, numerous fairly large spherical to oblong chromatoph
pyrenoids, numerous small paramylum, and with dimensions of 55–81 ×
could probably be a form of *E. orientalis*.

E. orientalis could probably be placed near *E. proxima* under Grou
because of its fairly large spherical chromatophores without pyrenoid
paramylum which are small or fairly large, its metabolic cell and the prese
pointed tail.

Distribution in Indian region: Pakistan (Kashyop 1908); Andhra Pradesh
figure 15) and Orissa (!). In Naidu's organism (60–91 × 18–23 μm) the
hores and paramylum are figured as small and discoid and the nucleus
spherical one.

Euglena elastica Prescott 1944 (figure Ee)

Gojdics 1953, p. 96, plate 10, figures 3a–b; Huber-Pestalozzi 1955,
106B.

Pradesh. His specimens measured 70–75 × 10–14 μm. He states that the p
are absent. It is possible that he could not distinguish the paramylum
chromatophores.

This species can probably be put under Group Lentiferae near *Euglena*
(Bhatia) Huber-Pestalozzi because of its highly metabolic cell and the nume
chromatophores and paramylum besides an ellipsoid median nucleus.

Distribution in Indian region: Andhra Pradesh (Naidu 1962); Karnataka (D
al 1973; Hosmani and Bharati 1975; 1980); Orissa (!).

Euglena clara Skuja 1948 (figure Ec)

Skuja 1948, p. 190, Tafel 22, figures 12–16; Pringsheim 1956, pp. 84–85,

Cell slightly metabolic, elongate oval to nearly hexagonal with almost par
anterior and posterior ends rounded, the latter end being sometimes slightly
periplast faintly striated spirally; reservoir fairly wide with gradually narro
which opens towards one side of the cell; eye-spot prominent; flagellum a
length or slightly shorter or longer; chromatophores eight or more po
irregular discs with prominent double-sheathed pyrenoids; nucleus sphe
median or slightly below; cell 36–40 × 17–19·5 μm.

Habitat: In plankton of location 28 (May).

The organism agreed fairly well with Skuja's and Pringsheim's specimens.
the cells were not as elongate as in their material and resembled the
individual figured by Pringsheim (figure 20D). Skuja gives the dime
35–68 × 15–19 μm. In Pringsheim's material the most frequent sizes were
15–17 μm. The smaller size in the author's collection was probably be
specimens were juvenile forms freshly liberated from the cyst.

Pringsheim puts this species near *E. polymorpha* Dangeard (1901), und
Catilliferae.

Distribution in Indian region: W. Bengal (!).

Euglena vaginicola Philipose 1982

Euglena srinagari (Bhatia) Huber-Pestalozzi 1955
See Philipose 1982, pp. 577–80
Under Distribution, omit Kerala and add Tamilnadu.
Euglena granulata (Klebs) Schmitz 1884
See Philipose 1982, pp. 582–83, figure 17.
Figure Eg shows figure 17 in Philipose (1982) redrawn with the striae fro
Figure Eg1 is another typical individual.
Euglena sanguinea Ehrenberg 1830
See Philipose 1982, pp. 592–93
Under Distribution, add Andhra Pradesh.

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)rigin and evolution of tetraploid forms within the 'olanum nigrum L. complex

P V BHIRAVAMURTY and P RETHY
Department of Environmental Sciences, Andhra University, Waltair 530003, India

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Abstract. Autotetraploid of *S. americanum* (D1-col) was compared with the dipl progenitor, *S. americanum* Mill (= *S. nigrum* 2*x*) (D1) and the natural tetraploid, *S. villos* Mill (= *S. nigrum* 4*x*) (T1). The study based on morphological comparison, *t*-test interpopulation differences in mean values, discriminant analysis, D^2 analysis, amino ac flavonoid- and sugar-analyses clearly pointed out that autotetraploid has more affinity w the natural tetraploid than with its diploid progenitor leading to the conclusion t autopolyploidy might be significant in the evolution of tetraploid forms within the *S. nig* complex.

Keywords. *Solanum nigrum* complex; polyploidy

Introduction

ne Indian tetraploid nigrum, which is now allotted the specific epithet, *S. villosum* N Rao 1964; Edmonds 1977, 1978), and the hexaploids have often been regarded lopolyploids (Bhaduri 1945; Tandon and Rao 1973). Recently Singh and Roy (19 ggested that the naturally occurring polyploids of the *S. nigrum* complex could topolyploids, though their chromosomal behaviour is different. Their observatio owever, pertained mainly to hexaploids and higher ploidy levels. However, su nflicting reports necessitate further probe into the aspect. The present study w erefore, taken up to provide additional clues in understanding the aforesaid proble

Material and methods

eeds of the diploid (D1) and tetraploid (T1) forms of the *S. nigrum* complex collect cally were grown in the Botany Experimental Farm. Autotetraploidy was induced

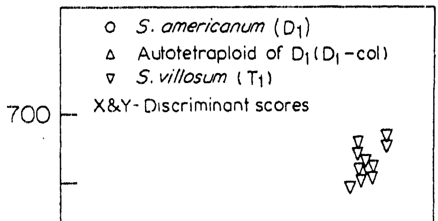
Table 1. Comparison of morphological characters in D1, T1 and D1-col p
S. nigrum complex.

Character	D1 (I)	T1 (II)	D1-col (III)	III
Habit	Erect	Erect	Erect	
Height (cm)	65·54 ± 3·38	52·60 ± 3·05	67·30 ± 3·24	1·7
Stem	Smooth green and cylindrical	Smooth tinged with anthocyanin and ribbed	Smooth green and cylindrical	
Leaves	Ovate, dentate, glabrous and thin	Ovate, slightly dentate, glabrous and thick	Ovate dentate glabrous and thick	
Lamina length (cm)+	5·96 ± 0·14	5·58 ± 0·11	5·32 ± 0·14	0·6
Lamina breadth (cm)+	3·29 ± 0·09	4·12 ± 0·11	4·26 ± 0·13	0·9
Lamina thickness (μ)+	208·60 ± 4·15	260·60 ± 6·01	246·40 ± 0·02	37·8
Stomatal length (μ)+	21·75 ± 0·39	21·55 ± 0·58	31·10 ± 0·61	9·3
Stomatal breadth (μ)+	13·90 ± 0·26	15·00 ± 0·25	20·65 ± 0·41	6·7
No. of stomata/unit area+	15·70 ± 0·25	7·20 ± 0·73	8·80 ± 0·55	6·9
Petiole length (cm)+	0·82 ± 0·03	1·10 ± 0·05	0·46 ± 0·02	0·3
Peduncle length (cm)+	1·41 ± 0·05	1·13 ± 0·03	1·76 ± 0·10	0·3
Pedicel length (cm)+	0·61 ± 0·01	0·83 ± 0·02	0·61 ± 0·01	0·0
No. of flowers/inflorescence+	3·76 ± 0·16	4·82 ± 0·17	3·56 ± 0·17	0·2
Corolla diameter (cm)+	0·65 ± 0·01	0·92 ± 0·01	0·90 ± 0·01	0·2
No. of fruits /inflorescence+	3·34 ± 0·18	4·24 ± 0·19	2·68 ± 0·17	0·6
Diameter of fruit (cm)+	0·61 ± 0·01	0·67 ± 0·01	0·44 ± 0·02	0·1
No. of seeds/fruit+	35·16 ± 0·96	23·94 ± 0·72	10·70 ± 1·45	24·4
Fruit colour	Purple black	Orange red	Purple black	
Pollen stainability	95·5%	93·7%	17·2%	74·3
Pollen diameter (μ)+	23·40 ± 0·61	30·70 ± 0·38	26·10 ± 0·61	2·7
Weight of 100 seeds (g)	0·0405	0·0910	0·0630	0·0
Sclerotic granules	Absent	Present	Absent	
Seed germination	100%	96%	20%	80
Chromosome No. (2n)	24	48	48	24
Meiosis	Regular	Regular	Irregular	
mg chlorophyll a/g tissue	0·7357	0·7138	0·9730	0·0
mg chlorophyll b/g tissue	0·9774	0·8188	1·4378	0·1

discriminant coefficients, discriminant functions and D^2 values between
populations are given in table 2. Discriminant graph was plotted using
discriminant functions as co-ordinate axes (figure 1). Amino acid analysis of D
D1-col was carried out using the seed extracts as suggested by Dunnil and
(1965). Identification of the amino acids was based on the Rf values of the

Table 2. Pairwise discriminant analysis of natural D1, D1-col and T1 populatic
nigrum complex.

	Discriminant coefficients				
Characters	D1 *vs* T1		D1 *vs* D1-col		T1
Leaf length/breadth	12·35	5·48	12·82	4·31	26·2
Leaf thickness	0·18	0·24	0·04	0·08	0·0
Peduncle length	25·53	22·64	3·28	4·92	7·9
Pedicel length	67·49	95·57	104·30	102·27	112·0
No. of flowers inflorescence	3·91	5·70	4·48	4·28	9·4
Corolla diameter	193·56	258·08	128·64	177·19	237·8
Pollen diameter	2·09	2·85	1·95	2·25	5·3
No. of fruits/ inflorescence	−1·22	−1·12	0·87	1·00	2·4
Fruit diameter	136·91	148·61	73·97	68·39	117·8
No. of seeds/fruit	1·07	0·83	0·18	−0·20	−0·1
Discriminant function of					
D1	442·86	—	300·85	—	—
T1	—	639·54	—	—	659·1
D1-col	—	—	—	361·82	—
D^2(D)	1033·07	(32·14)	745·12(27·30)		550·3



amino acid run concurrently (table 3). The qualitative data were quan
method of Ellison *et al* (1962). Paired affinity (PA) and group affinity
given in table 4. One-dimensional paper chromatography of fruit wall
acetic acid along with marker solutions aided in separation of flavon
1973). The dried chromatograms were observed under UV lamp to l
spots. Identification of flavonoids was based on the marker spots develo
it (table 5). One-dimensional paper chromatography of the same extr
pyridine-water (6:4:3) solvent system and subsequent spray with am
nitrate revealed the presence of a total of 4 different sugars in fruit wall
populations (table 6).

3. Observations

D1-col resembles D1 externally in every respect but for the thicker and
closer examination reveals that D1 differs from D1-col in almost all char

Table 3. Amino acids in seeds of D1, T1 and D1-col populations of the *S. nigrum* complex.

Amino acid	D1	T1	D1-col
Alanine	+	+	+
Aminobutyric	+	+	+
Asparagine	+	+	+
Aspartic	+	+	+
Glutamic	+	+	+
Glycine	+	+	+
Leucine	+	+	+
Iso-leucine	+	+	+
Lysine	−	+	+
Methionine	+	+	+
Phenylalanine	+	+	+
Proline	+	+	+
Serine	+	+	+
Threonine	+	+	+
Tryptophan	+	+	+
Tyrosine	−	+	+
Valine	+	+	+
Unidentified spots			
A	+	+	+

Table 5. Flavonoids detected in the fruit wall extracts of D1, T1 and D1-col populations of the *S. nigrum* complex.

Populations	Flavonoids		
	Quercetin	Myricetin	Unidentified
D1	+	+	−
T1	+	−	−
D1-col	+	+	+

Table 6. Sugars present in the fruit wall extracts of D1, T1 and D1-col populations of the *S. nigrum* complex.

Populations	Sugars			
	Glucose	Mannose	Rhamnose	Glucuronic acid
D1	+	+	+	+
T1	+	+	+	+
D1-col	+	+	−	+

like plant height, leaf-shape, -margin and -texture, pedicel length, and nu flowers per inflorescence (table 1). D1-col can also be easily distinguished fro the colour of the ripe berries which is purplish black in the former and orange r latter. Berries of T1 contain sclerotic granules while those of D1-col and D1 la Meiosis is regular in D1 and T1 but is irregular with multivalent and u formation in D1-col. The meiotic irregularity is also reflected in the low perce pollen stainability and the reduced seed germination percentage. Chlorophyll of D1-col leaf is more compared to that of either D1 or T1. The *t*-test shows population pair T1 *vs* D1-col differs significantly in ten characters while the pa D1-col shows significant differences in 12 out of the 14 characters tested (ta

Discriminant analysis revealed that pedicel length, corolla diameter a diameter are the three characters that contribute most to the discrimination of t taxa under study (table 2). Closer affinity of D1-col with D1 is brought out

4. Discussion and conclusion

Indian diploid *Solanum nigrum*, which most probably is a geogra *S. americanum* (Rao 1964; Edmonds 1977, 1979b; Rao 1978; Schilli tinguished from the tetraploid *S. villosum* (= *S. nigrum*) primarily o ripe berry colour which is purple-black in the former and orange-red in is a preponderance of evidence in support of the allopolyploid nature forms. Emphasis is laid on the fact that autotetraploids bear purple-b show irregular meiotic behaviour and low pollen and seed fertility (Bhi Rao 1964; Tandon and Rao 1966, 1973; Chennaveeriah and Patil 1968 and Rao 1972; Edmonds and Glidewell 1977; Edmonds 1977, 1978, 19 (1965), Schilling and Heiser (1976), Heiser *et al* (1979) and Schill reported that their numerical and biosystematic studies helped in di three ploidy levels. Autoploids tended to cluster closely with their dip acquiring distinction only by their polyploid nature but not d differentiation as in other alloploid hybrids. Discriminant analysis patterns of the present study also suggested that D1-col is closer progenitor than to the natural tetraploid, T1, thereby supporting th natural tetraploids may not be autotetraploids.

The presence of anthocyanins in the purple black berries of carotenoids in the orange-red berries is now a well-established fact inheritance study of the fruit colour of the complex has led to the different allelles of the same locus are responsible for this difference ar colour was inherited as dominant over the orange-red (Venkateswarlu This being the case, mere presence of anthocyanins in the autotetraploi rule out the autopolyploid nature of the tetraploids. A mutation can exp which, of course, must await experimental verification. Meiotic ir pollen and seed fertility are ubiquitous features associated with any colchiploids. A detailed study of the subsequent generations of colchip the natural polyploids is worthwhile to throw light on the nature of p the complex.

Singh and Roy (1982) stated that autopolyploidy has played a m evolution of polyploids of the complex particularly at the hexaploid levels. The present observation shows that the three taxa are dissimila morphological characters (table 1). But dissimilarity between D1-col a

that variable *S. americanum* might have contributed a genome to *S. villosum.* S the seed protein band patterns suggested that among the diploids most like been involved in the ancestry of *S. villosum* are *S. sarrachoides* and *S. su* (Edmonds and Glidewell 1977). Non-hierarchic clustering pattern based on morphological attributes strongly supported the participation of *S. sarrachoi* also implicating *S. sublobatum* in the evolution of *S. villosum* (Edmonds 197 This conclusion does not seem to hold good for the Indian races, as both th progenitors suggested are not known to occur naturally in the subcontinent. races of *S. americanum* (= *S. nigrum*) might have contributed to the evo different morphological races of the Indian tetraploid *S. nigrum* (= *S. vill*

On the basis of the aforesaid discussion it is concluded that autopoly significant in the origin and evolution of *S. villosum.* Lack of distribut *sarrachoides* and *S. sublobatum* in the subcontinent casts doubt on their invol the ancestry of the Indian tetraploid races.

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Mucilage interference in desmids under SEM

VIDYAVATI and JOHN D DODGE*

Botany Department, Kakatiya University, Warangal 506 009, India

*Department of Botany, Royal Holloway College, London, UK

MS received 26 August 1983; revised 26 June 1984

Abstract. Various species of desmids (*Cylindrocystis brebisonii, Closterium* *Closterium littorale, Cosmarium bioculatum, Cosmarium botrytis, Cosmarium contr* *Cosmarium subtumidum*) were studied for their surface ornamentation. After tryi methods for removal of the mucilage, it was found that pretreatment with th preparation was usually effective in cleaning the cells and enhancing their appear the SEM.

Keywords. Desmids; *Cylindrocystis*; *Closterium*; *Cosmarium*; Scanning electron n

1. Introduction

In Desmidiaceae, the cell wall consists of two layers a thin inner layer c mainly of cellulose and an outer firmer and thicker layer composed of impregnated with pectic substances which often include iron compounds. Ex the outer layer is a narrow/broad diffluent mucilage layer. The mucilage is through a large number of pores which traverses both the layers. Some pl desmids have a wide mucilage envelope. Pores were not found in the walls of a Mesotaeniaceae, although there is a copious production of mucilage by these (figure 1).

At the ultrastructural level Pickett-Heaps (1972) showed that, irregula material usually remained attached to the external orifice of the pore, and i there is always a prominent hemispherical invagination of the plasmalemma co strands of mucilagenous material.

The mucilage produced from the pores of *Cosmarium* is composed of s polysaccharides. This mucilage is often very difficult to remove completely and


1
3

2. Material and methods

Cylindrocystis brebisonii Menegh, 615/1a; *Closterium acerosum* Ehre
Closterium littorale Gay, 611/6; *Cosmarium bioculatum* Breb, 612/17;
botrytis Menegh, 612/5; *Cosmarium contractum* Kirchn, 612/16; and
subtumidum Nordst, 612/8c (cambridge culture collection, UK) species were
in their unialgal clonal form. The cultures were grown in Chu's 10 inorgan
receiving 16 hr light and 8 hr dark period at 18–20°C.

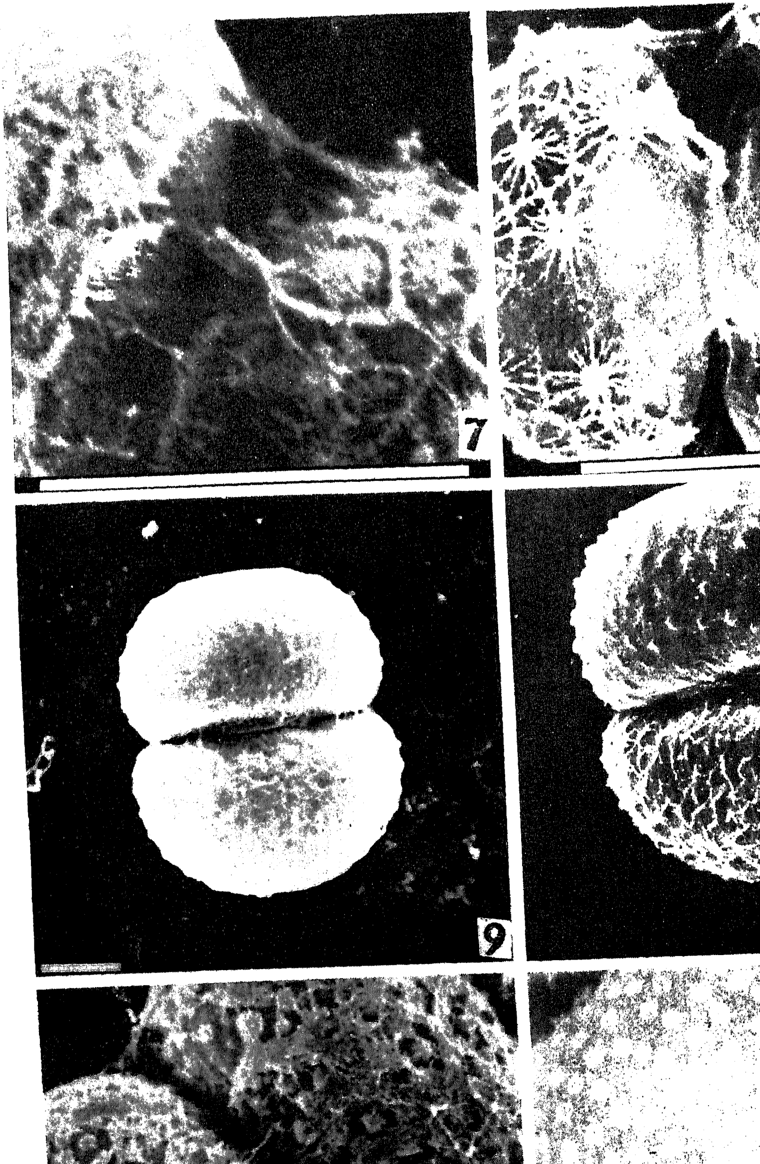
All cells prepared for SEM were washed in filtered water before processing.
cells characteristically secrete various quantities of mucilagenous mat
secretions can undoubtedly be considered as an integral component of the c
pose problems because they condense during dehydration into a fibrous or
layer, partially or totally obscuring the surface of the cell or its true cell-

Some mucilaginous layers are soluble in acetone-water or alcohol-wat
and were therefore extracted in the course of dehydration. Warm water (30
without chelating agents (*e.g.*, 0·02 M EDTA) solubilizes pectic fractions of h
cells and are sometimes successful in removing surface layers from algae. Al
(50 °C) or cold (0 °C) solutions of sodium hydroxide (0·01 N) are mo
extractive their use caused an increasing likelihood of damaging the cell-v

Certain wall-degrading enzyme preparations (*e.g.*, snail gut juice, pecti
also used for removing mucilage. Acid solutions were also tried but w
avoided except for organisms with siliceous walls or scales. Few cells were
dilute alkali, but a majority remained completely enshrouded in their mu

Frequent subculturing also helped to remove mucilage. Thus only fre
cultures were fixed for the SEM studies. Pickett-Heaps (1973) and Marc
developed methods that have been used successfully with many small
Hydrodictyon, *Pediastrum*, *Cosmarium*, *Staurastrum*). But incubation of spe
solution of the polysaccharidase preparation of glusulase (Endo Laborat
York) was definitely better than all other previous treatments. The glusulase
1:50 (v/v) dilution in distilled water for 1½–2 hr at room temperature, v
allowed to stand at room temperature for 1 hr in culture medium before

After pretreating with glusulase, the cells were fixed in glutaraldehyde, p
osmium tetroxide and then dehydrated with varying grades of acetone (30
and 100 %). The cells were then dried (critical point-drying apparatus), c
carbon and gold and finally observed under SEM (JEOL-JSM-25S)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the action of glusulase in removing the mucilage from the cell surface. T
treatment is quite efficient compared to all other methods tried.

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Development of the caryopsis in *Chionachne koenigii* Lin

T V CH SATYAMURTY

Department of Botany, Andhra University, Waltair 530 003, India

Present address: Government Junior College, Erragondapalem 523 327, In

MS received 13 February 1984; revised 2 July 1984

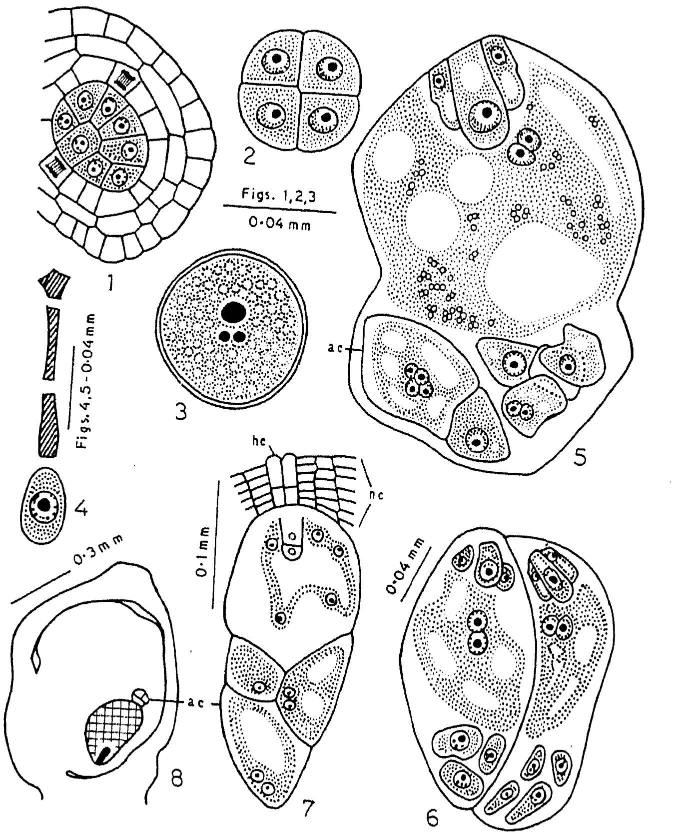
Abstract. The anther wall of *C. koenigii* showed the epidermis, endotheci and tapetum. Cytokinesis in the pollen mother cells was successive and iso tetrads were formed. The mature pollen grains were three-celled. The dev embryo sac conformed to the Polygonum type. The antipodal cells increase persisted in the young fruit. Twin embryo sacs occurred in about 30% Endosperm development was nuclear.

Keywords. *Chionachne koenigii*; Maydeae; Panicoideae; Poaceae; caryopsi

1. Introduction

The sub-family Panicoideae of the Poaceae includes a number of impo which have both forage and food value. Investigations of Narayanaswam 1955a, b; 1956), Koul (1959), Chandra (1963) and Shanthamma (1979) h our knowledge of the embryology of this sub-family, while investigations Emery (1958) have established the occurrence of apomictic phenomena ir species. Although appreciable embryological information is available Maydeae of the Panicoideae (Koul 1959), *Chionachne koenigii* has not attention of embryologists. This paper deals with the more important deta embryological aspects leading to the development of the caryopsis in

2. Material and methods



Figures 1–8. Development of the caryopsis in *C. koenigii*. **1.** TS of an anther
microspore mother cells and wall layers. **2.** Microspore tetrad. **3.** Three-celled
4, 5. Linear tetrad and organised embryo sac. **6.** Twin embryo sacs. **7.** Nuclea
8. LS of young caryopsis showing persistent antipodal cells.
Abbreviations: *ac*, antipodal cells; *hc*, hypertrophied cells; *nc*, nucellar cap.

3.2 *Nucellus, megasporogenesis and female gametophyte*

The nucellar epidermis at the apex of the ovule underwent one or more

Initially there were three, prominent, uninucleate antipodal cells. The antipodal ce
vided to form 4–8 cells in a mature embryo sac (figure 5). The nucleus of each of t
ntipodal cells divided without wall formation and as many as 10 nuclei could
ounted in a cell. The antipodal cells persisted for a considerable time (figure 8).
bout 50 ovules examined 30% of the ovules showed twin embryo sacs (figure 6)

3 *Post-fertilisation changes in the ovary and ovule*

he primary endosperm nucleus underwent rapid divisions and the nuclei formed we
stributed in the peripheral cytoplasm enclosing a central vacuole (figure 7). Cell w
rmation in the endosperm began in the region around the proembryo and la
xtended towards the antipodal end. By the time embryonal organs were differentiat
e peripheral layer of the endosperm formed the aleurone layer.

The structure of the mature caryopsis wall was similar to the one described for whe
Esau 1974).

Discussion

win embryo sacs now observed in *C. koenigii* were reported earlier in *Tripsacu
actyloides* (Farquharson 1955) and *Euchlaena mexicana* (Koul 1959). Such embry
cs may have developed from the functioning of two megaspore mother cells.

The three antipodal cells divide and form a varying number of cells. Such an increa
the number of antipodal cells were reported in grasses previously (Swamy 194
hikkannaih and Mahalingappa 1975; Muniyamma 1976). Khosla (1946) record
e presence of 23 nuclei in the antipodal cells of *Pennisetum typhoideum*. T
ntipodal cells remain at the basal end of the mature embryo sac as in other members
e sub-family Panicoideae (Chandra 1963). Weatherwax (1926) and Randolph (193
ave observed the persistence of antipodal cells in the mature caryopsis of *Zea may*
arayanaswami (1955b) reported that in *Echinochloa frumentacea* the persiste
ntipodal cells might be involuted endosperm cells rather than real antipodal cells. T
resent study on *C. koenigii* reveals them to be undoubtedly antipodal cells.

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eed germination, seedling growth and haustorial induction Santalum album, a semi-root parasite

ARCHNA SAHAI and K R SHIVANNA
Department of Botany, University of Delhi, Delhi 110 007, India

MS received 25 May 1984; revised 29 August 1984

Abstract. Seed germination and early growth of the seedling in *Santalum album*, a semi-r parasite, is independent of the host but seedling establishment seems to be dependent on establishment of host contact. About half of the seedlings, raised in aseptic cultures, show drying of the shoot tip upon transfer to fresh medium and subsequent development of a la number of adventitious shoot buds on the hypocotyl. Attempts to induce rooting in th shoot buds were unsuccessful. Gum tragacanth, which induces haustoria in t Scrophulariaceous root parasites, and xenognosin, the active fraction from gum tragacan were effective in inducing haustoria in *S. album* in the absence of the host.

Keywords. *Santalum album*; sandal wood; semi-root parasite; seed germination; seedl establishment; adventitious shoot buds; haustorial induction.

Introduction

ntalum album (Santalaceae) is an arborescent semi-root parasite native in Sou dia, but planted elsewhere in some tropical regions. It is the source of an essential ndal wood oil. Although several studies have been carried out on the morphogene tentialities of embryo, endosperm and hypocotyl segments in aseptic cultu angaswamy and Rao 1963; Rao 1965; Rao and Bapat 1978; Bapat and Rao 19 84; Lakshmisita 1979; Lakshmisita *et al* 1979, 1980), experimental studies on se rmination and seedling establishment are very limited (Srimathi and Rao 19 agaveni and Srimathi 1980, 1981). Such studies are useful not only in understandi e biology of the parasite, but also in its propagation through seeds, which is so f e only method available for this species. We have investigated the requirement ed germination, seedling growth and haustorial development and this paper repo e results.

Whatman No. 1 filter paper moistened with 9 ml of distilled water. In
fruits were washed in running tap water for 1–6 days before surface
culture. In another experiment, the fruits were scarified by trea
concentrated sulphuric acid for 5–25 min, washed in sterile water an
germination.

To raise seed cultures, the fruit wall was manually removed. Subseq
surface-sterilized and sown for germination in the same way as des
fruits. As the seeds took over 14 days for germination, petriplate cul
infected. Studies were, therefore, largely carried out in aseptic cu
Nitsch (1951) basal medium was used. In one experiment the ef
carbohydrate sources (sucrose, glucose, xylose, mannose and gala
casein hydrolysate, on seed germination and seedling growth was t

In petriplate cultures three replicates of 25 fruits/seeds each were
treatment. In aseptic cultures, 24 fruits/seeds were used for each tr
experiment was replicated at least once. The cultures were maintai
under diffused light conditions. One set of cultures was maintained

The effect of many growth regulators—gibberellic acid (GA_3),
purine (6-BAP), kinetin (Kn) and thiourea (TU) (added singly at
ranging from 10^{-6} to 10^{-4}M) and ethrel (50–400 μg/ml) were studied
of cultured fruits and seeds.

The seedlings raised in aseptic cultures were transferred to fresh med
days of culture. On transfer, some of the seedlings showed drying
followed by development of shoot buds on the hypocotyl. To induce
buds were excised and transferred to fresh medium. Apart from Nitsc
(1951), Murashige and Skoog's medium (1962), Tepfer's medium (Te
and White's medium (1963) supplemented with various hormones (i
(IAA), indolebutyric acid (IBA), naphthaleneacetic acid (NAA), 6-benzyl
BAP)) were also tried.

Aseptically grown seedlings were transferred to the soil. Some of th
transplanted to pots in which seedling of *Lantana camara* L., a na
growing. The seedlings were maintained under laboratory/field con

For induction of haustoria, the efficacy of gum tragacanth (an exud
a legume—*Astragalus* sp.) and 'xenognosin' (a host recognition fact
gum tragacanth) which are effective in inducing haustoria, in two Sc
root parasites, were tested.

Aqueous extract of gum tragacanth (Sigma) was prepared by boil

e hypocotyl and of haustoria induced in the absence of host. The material was fix 10 % aqueous acrolein for 24 hr at 0°C and dehydrated through a 2-metho hanol–ethanol–*n*-propanol–*n*-butanol series (Feder and O'Brien 1968). T aterial was then infiltrated and embedded in JB_4 resin (Polysciences USA). Sectio μm thickness) were stained with toluidine blue (Merck) (0·1 %) in 0·1 M acet uffer, pH 4·5 (Feder and O'Brien 1968), mounted in DPX mountant (BDH) a oserved.

Observations

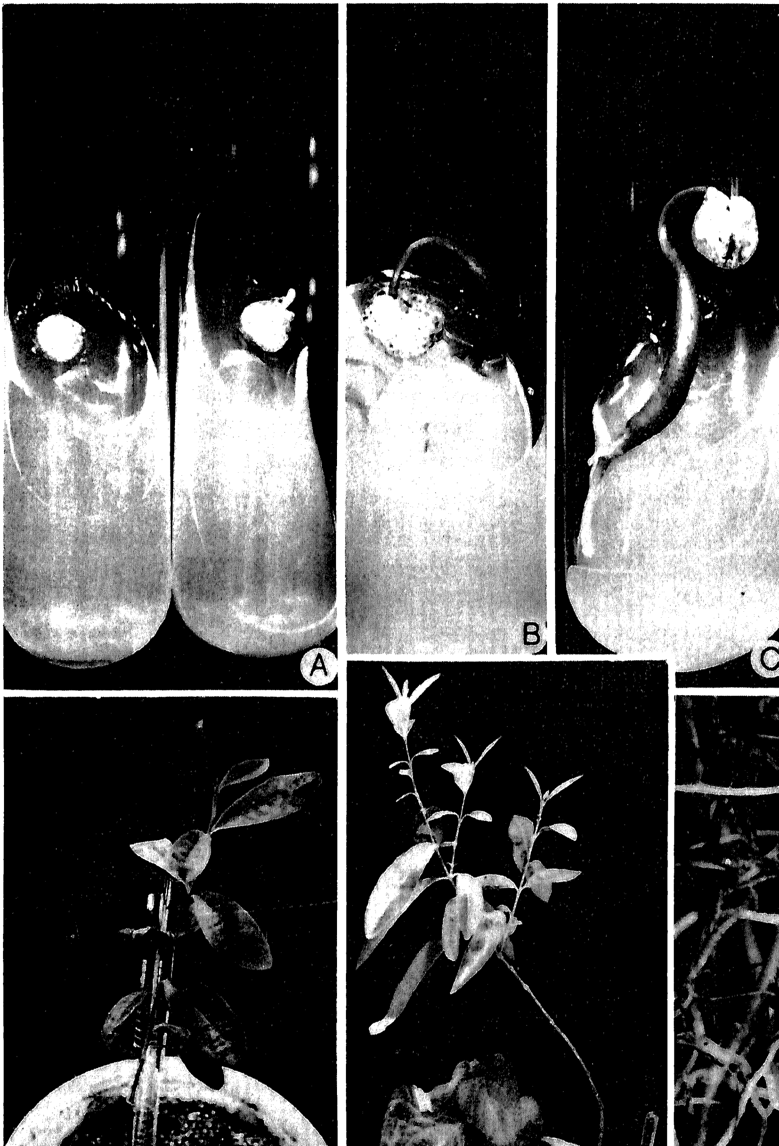
1 *Fruit cultures*

he fruits failed to germinate both in aseptic and petri plate cultures. Neither wa ashing nor scarification could induce germination. The scarified fruits show ydration and splitting of the fruit wall after 20 days of incubation but no germinatio milarly treatment with chemicals such as GA_3, 6-BAP, Kn, TU and ethrel failed duce germination under both light and dark conditions.

2 *Seed cultures*

eds readily germinated in petriplate as well as in aseptic cultures. There was no ne r any pre-treatment. Agar (0·6 %) medium alone supported germination of up out 40 % seeds. Addition of mineral salts, vitamins, and amino acids (basal mediu) and sucrose improved germination only up to about 50 %. Light was fou sential for germination. Following hydration a small protuberance appeared on o le of the decoated seed in about 10 days. In another two days, the protuberan owed a splitting (figure 1A). Almost all the seeds showed these responses on all rmination media tested. In many of the seeds, however, germination did not proce yond this stage. In others, radicle emerged in about 14 days of culture and form oolly root hairs near the tip. Subsequently the hypocotyl elongated with a promine ching (figure 1B) while the cotyledons still remained enclosed within the endospen ventually the hypocotyl became erect, lifting the cotyledons and the surroundi dosperm from the surface of the medium (figure 1C). Gradually the endospe ied and the cotyledons unfolded. The shoot produced 3–5 pairs of leaves in abo ree months (figure 1D).

Amongst different carbohydrates (added singly to BM) tested, xylose, mannose, a


A
B
C

3.2a *Transplantation of seedlings*: Three-month-old seedlings grown o medium were transferred onto the fresh medium in 250 ml flasks. The se continued growth and reached up to the cotton plug in about one month. The se were then transferred to plastic pots containing sterilized vermiculite (figure maintained in the culture room under diffuse light for one month. They were i with distilled water or Hoagland and Arnon's (1950) nutrient solution. The gr distilled water was as good as in Hoagland and Arnon's solution. Subsequent were transferred to autoclaved garden soil in pots and maintained in the cultur The seedlings survived in pots in the culture room for up to about eight month could not be grown under laboratory conditions as they started drying in ab weeks after shifting.

Some of the seedlings from vermiculite were transferred to garden soil together with a seedling of *Lantana camara* and maintained in the laborator seedlings survived the transfer and (figure 1F), produced many new branches. them which was subsequently kept under field conditions (in shade) continued When plants were carefully uprooted and washed, after about 15 months, *S* roots showed a large number of haustorial connections with the host roots (fig

3.2b *Origin of adventitious shoot buds*: On transferring to fresh medium, the about 60 % of the seedlings ceased growth and the shoot tip started drying. Th extended towards the base up to the level of the cotyledons (which generally by the time 3–4 pairs of leaves develop) (figure 2A). Such seedlings development of many protuberances on the surface of the hypocotyl just be dried shoot tip in 10–15 days of transfer. Subsequently, these protuberances de into normal shoot buds (figure 2B, C). Such shoot buds developed only upon of the seedlings and never in the original cultures maintained for many months transfer.

The shoot buds originated through the activity of subepidermal cortical cell cells became meristematic, and produced a compact meristemoid (figure 2 resulted in the development of protuberance on the epidermal surface (fig Shoot apex soon differentiated at the tip of the meristemoid facing the ep Shoot primordia emerged by rupturing the epidermal layer.

Anatomically the shoot buds had normal shoot apex (figure 2F, G). vascular strands of the shoot bud were not connected to the vascular bundle axis. On subsequent growth these vascular strands established contact with tho main axis. Out of the large number of shoot buds that were initiated earlier, onl


A
B
D

The sections showed the mantle, the collapsed layer and the central body (fig

Xenognosin was also effective in haustorial induction. In 20 % of the cult roots became swollen only at the highest concentration (30 μg/ml) of xen tested. These swellings were similar to those formed on the gum trag supplemented media. When such seedlings were transferred to pots co vermiculite, many haustoria as well as autohaustorial connections developed four weeks (figure 3C).

4. Discussion

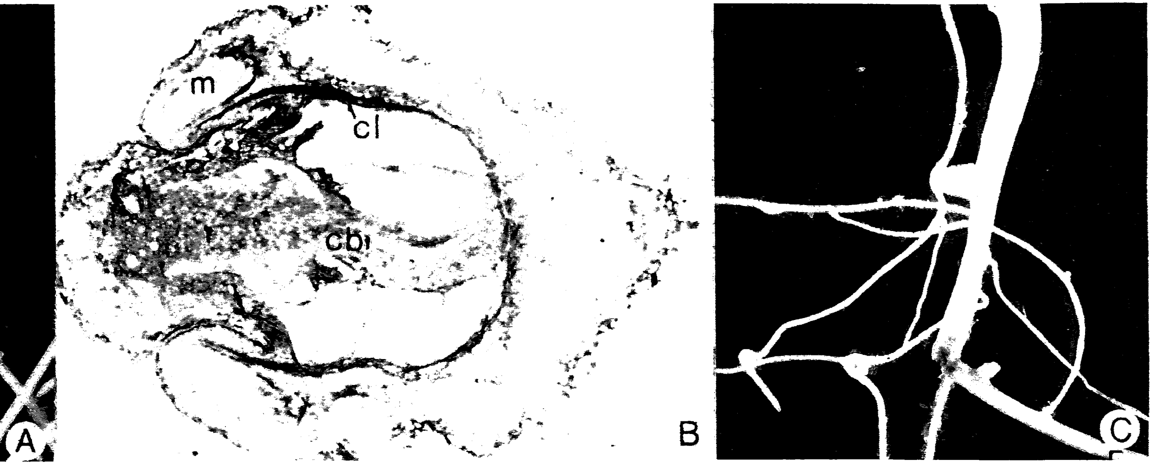
Using aseptic culture, Rangaswamy and Rao (1963) reported germination only in the presence of casein hydrolysate or coconut milk and suggested that may require some stimulus from the host roots. Subsequently, Srimathi a (1969), however, obtained germination of seeds both in petriplate cultures an without any pretreatment or addition of any growth substances. Germinatio entire fruit was, however, not satisfactory.

In the present investigation also entire fruits failed to germinate where germinated readily without any pretreatment or addition of any stimulant from acting as a barrier for hydration, the fruit wall also seems to conta inhibitors. Scarification of fruits with concentrated H_2SO_4, although hydration of seeds, failed to promote germination.

Seeds germinated readily without any pretreatment and produced normal s Light was essential for germination. Unlike many other root parasites, in S *album* it appears that seed germination and early seedling growth are indepe the host stimulus. It requires neither a carbohydrate source nor any exogenou substance. The seeds contain a massive endosperm filled with food material t seedling growth until the development of leaves. Seedling growth could be ma for many months without providing a carbohydrate source in the medium.

When the aseptically grown seedlings were transferred to soil, they could no for more than a few months. However, when transferred in the vicinity of a seedling readily established haustorial contact with the host and continued Thus for a successful establishment of the seedling in soil, development of ha contact with host root seems to be necessary.

An intriguing observation in the present study is the drying up of the shoots 50 % of the seedlings upon transfer to fresh medium, and subsequent developn large number of adventitious shoot buds. The development of adventitio



l induction. **A.** Gum tragacanth-induced autohaustoria. **B.** Off median LS of autohaustoria passing through one of the roots
sed layer (cl) and central body (cb). **C.** Xenognosin-induced autohaustoria.

Because of the importance of the sandalwood tree in essential oil ind attempts have been made to obtain clonal multiplication through tissue and Bapat 1978; Bapat and Rao 1979; Lakshmisita *et al* 1979, 1980) segments, shoot segments and shoot tips have been used, and ca differentiation of embryoids as well as shoot buds have been achieved in th Plantlets have been readily obtained from embryoids. However, inductio the shoot buds has not been very successful. Rao and Bapat (1978) report a few cultures on a medium containing NAA and IBA. In the present i rooting could not be induced in shoot buds although as many as 85 com different growth substances including those used by Rao and Bapat (197 Induction of rooting in these shoot buds, would greatly facilitate clonal As there is no callusing in the production of shoot buds, the feasibility of o clones through this method is more than that through embryoids.

The parasitic behaviour of *Santalum* has been described by Barber ((1935) and Rao (1942). Until recently there were no experimental studies induction in any root parasitic taxa. Studies during the last few years (N 1979; Riopel and Musselman 1979; Lynn *et al* 1981; Sahai and Shivanna have shown that gum tragacanth is effective in inducing haustoria in *Agal* and *Sopubia delphinifolia* both of the Scrophulariaceae. Subsequentl fraction from gum tragacanth was isolated and characterized, and 'xenognosin' (Lynn *et al* 1981).

Both gum tragacanth and xenognosin were effective in inducing *Santalum album* also. Haustorial inducing factor in *S. album*, therefore, the same or very much similar to that effective in *Agalinis purpurea* and *S.* Induction of haustoria in the absence of host would facilitate experiment the details of host recognition and haustorial differentiation.

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he fern family *Elaphoglossaceae* Pichi-Sermolli in India, epal and Bhutan

A BISWAS and S R GHOSH

Cryptogamic Section, Botanical Survey of India, Howrah 711 103, India

MS received 29 December 1983

Abstract. Taxonomic study of the family *Elaphoglossaceae* consisting of the gen *Elaphoglossum*, *Microstaphyla*, *Peltapteris* is done. Only the former genus exists in Inc Twenty species including ten which are new to science and two which are new to India described and their distributions are indicated.

Keywords. *Elaphoglossaceae*; *Elaphoglossum*, microstaphyla; peltapteris.

Introduction

though Schott (1854) proposed the name *Elaphoglossum*, Smith (1875), the auth the genus gives the epitomological origin of the name *Elaphoglossum*; elaphos mea ıg, glossa means tongue, *i.e.* stag's tongue. Since the establishment of the genus, stematic position has long been debated. Like all other botanists of the last centu ddome (1863, 1883); Clarke (1880) who largely followed Moore (1857) and Hool 364) thought it to be placed under the tribe *Acrosticheae*. Mehra (1961), on the ba cytological evidence placed the genus *Elaphoglossum* in *Aspidiaceae*. Pichi-Serm 968) after reviewing the historical accounts of systematic position, taxonomy a inities, validly published the family *Elaphoglossaceae* consisting of the gene *aphoglossum*, *Peltapteris*, *Microstaphyla* of which the latter two are non-existent dia, Nepal and Bhutan. No further elaboration of the treatment of the vario thors up to 1968 is required. Nayar (1970) in his classification thought it to be plac the family *Lomariopsidaceae*.

Crabbe *et al* (1975) placed *Elaphoglossum* along with *Peltapteris*, *Microstaphyla* subfamily *Elaphoglossoideae* under the family *Aspleniaceae*. Pichi-Sermolli (19 ter considering all the observations, again preferred to treat *Elaphoglossum* as ember of an independent family *Elaphoglossaceae* comprising of the two oth

3. Materials

This study is based on all the available herbarium specime
Elaphoglossum housed in various herbaria of Botanical Survey
specimens taken on loan from Forest Research Institutes, Dehrad
Botanic Garden.

4. Systematic accounts

Elaphoglossaceae Pichi-Sermolli, Webbia 23: 209, 1968; et in Webbi

This family consists of three genera *viz. Elaphoglossum,*
Microstaphyla of which the latter two do not exist in India. Theref
Elaphoglossum is described.

Elaphoglossum Schott ex J. Smith, Hook. J. Bot. 4(27): 148: 184
Hook. Gen. Fil. t. 105A, 1842; Moore, Ind. Fil. XVI, 1857 et 351, 18
Fil. 125, 1875; Bedd. Handb. Ferns Br. Ind. 416, 1883; Christ, Neu
Schweir. Ges. Naturw. 36: 1–159, 1899; Diels in E. & P. Nat. Pfl. Fa
C. Chr., Ind. Fil. 302, 1905; Holttum; Blumea 14: 317–326, 1966, et
vol. 1(4): 289, 1978; Mickel et Atehortua in Am. Fern. J. 70(2): 4

Olfersia Presl, Tent. Pterid. 232, 1836.

Aconiopteris Presl, Tent. Pterid. 236, 1836.

Acrostichum (non. L.) Fee, Hist. Acrost. 8, 27, 1845.

Dictyoglossum J. Sm. Bot. Mag. 72. Comp. 18, 1846.

Acrostichum sect. *Elaphoglossum* Hk. Spec. Fil. 5: 195–241, 1864
Syn. Fil. 400, 1868.

Lectotype: *Elaphoglossum comforme* (Sw.) J. Smith, Hook. J. B

Rhizome long, short creeping, erect, suberect, obliquely ascendi
upright. Aerophore present in some Indian species at the base of fr
lanceolate, ovate, thin, papery, glossy, apex of scale acute, acuminat
scale entire, or with short and long teeth or cilia, in some species cili
colour range from orange, brown and black, Frond: (i) in two rank
one on each side of the median line, (ii) crowded together in several
side of the rhizome or inserted all round the rhizome, dimorpho
scaly, scale similar or dissimilar to rhizome scale, in some species sti
of scale, stellate and lanceolate. Phyllopodia: present in most of

in endings are linked by a intramarginal commisure which may be continuous terrupted. Fertile frond longer or equal to sterile frond, lamina smaller, stipe long an in the sterile one. Lower surface covered with sporangia except at the marg ores monolete with smooth exine and smooth to granulose or even spinulose peri d variously ornamented.

stribution: About 450 species throughout the tropics and subtropics. Region und udy is represented by 20 species of *Elaphoglossum*.

Key to the species

Rhizome scale not glossy, frond scale minute, stellate, glandular or ovate w marginal teeth.

2. Surface scale stellate glandular
   3. Rhizome long creeping
      4. Lamina thin, translucent, vein evident, rhizome scale thick, bicolorous .... 1. *E. stigmatole*
      4. Lamina coriaceous, vein obscure, rhizome scale papery, thin, concolor .... 2. *E. angulat*
   3. Rhizome short creeping, erect, sub-erect
      5. Fronds in two rows on the dorsal side of the rhizome
         6. Lower surface scaly
            7. Apex of rhizome scale flexuose and twisted...... 3. *E. commutat*
            7. Apex of rhizome scale not flexuose and not twisted . . 4. *E. beddor*
         6. Both surface scaly
            8. Lamina lanceolate, texture thin.....................5. *E. thomso*
            8. Lamina ovate, texture coriacious ....6. *E. cherapu*
      5. Fronds in several rows on the dorsal side of the rhizome
         9. Stipe scales two types, stellate and ± lanceolate.....7. *E. meebo*
         9. Stipe scales one type, lanceolate or ovate
            10. Apex of rhizome scale hair-like not twisted ....8. *E. indic*
            10. Apex of rhizome scale not hair-like or twisted
                11. Rhizome scale attenuated.................9. *E. marginat*
                11. Rhizome scale otherwise
                    12. Fertile frond shorter than sterile one . . . 10. *E. fasciculat*

16. Rhizome scale black with black marginal teeth, lower surf
    moderately scaly ....16.

1. Rhizome scale glossy, frond scale distinctly stellate, not glandular

17. Scale on both surfaces adpressed 17

17. Scale on the upper surface pale adpressed, scale on the l
    ferruginous and loose

18. Surface scale with long slender hair

19. Scale with 3–5 marginal hairs spreading away in one direc
    .... 18.

19. Scale with 6–9 marginal hairs spreading away in all direct
    ....19.

18. Surface scale with short hair..........................20.

1. ***Elaphoglossum stigmatolepis*** (Fee) T. Moore, Ind. Fil. 16, 15, 18
Christ in Neue Denkschr. Schweiz. Ges. Naturw. 36(1, 2): 52. 1899; Sl
Mus. (Nat. Hist.) 4(2): 86, 1967.

Basinym: *Acrostichum stigmatolepis* Fee, Mem. Fam. Foug, 2: 62, t.
Hook. sp. Fil. 5: 216, 1864.

Synonyms: *Elaphoglossum conforme* Bedd. Fern. S. Ind. 67 t. 198, 18(
ferns Br. Ind. 416, fig. 247, 1883, non J. Smith
Elaphoglossum ballardianum Biswas in Bull. Misc. Inf. Kew 1939:
1939.

Rhizome horizontally long creeping, rigid (dia. 3–4 mm) densely cove
ovate lanceolate, attenuate, bicolourous, scale brown at base, bl
(3–5 mm long, 1–2 mm broad), margin entire, apex acute; fronds: dist

vo rows on the dorsal sides of the rhizome; Phyllopodia: distinct, swelled, bla
arrowed, 5–10 mm long, scaly; sterile frond: stipe twisted, deep brown, 8–12 cm lo
aly; Lamina: narrowly elliptic (10–15 cm long, 1·5–2 cm broad), black on dryi
ase narrowed and decurrent on stipe, margin entire, with narrow cartilagino
order, reflexed, apex narrowed, acuminate; costa scaly beneath, upper surface w
attered scales, Lower surface with minute dark, peltate scales with fimbriate marg
exture: thin, translucent; veins evident, once or twice forked, ends 0·5 mm bel
argin; hydathodes wanting; fertile frond: stipe longer than sterile one; Lami
arrowed, 1–2 cm broad.

pecimen examined: India: Tamilnadu: Ootkamond, Aug. 14, 1878, *G. King* s. n. (CA
Dodabetta, ± 2500 m, Nilgiri Dist. June 10, 1883, *H. C. Levinge* (CAL-2524
Aramby, ± 2200 m, Nilgiris, Sept. 1884, *Gamble* 15351 (CAL-25234); Nilgi
Beddome 159 (Type—CAL); Pandiar reserve forest, 2000 m, Nilgiri, Oct. 28, 1972,
L. Ellies 43425 (MH).

It grows on rocks or on trees.

istribution: South India; endemic.

Elaphoglossum angulatum (Bl.) T. Moore, Ind. 5, 1857; Sledge, Bull. Br. M
Nat. Hist.) 4(2): 83, 1967.

asinym: *Acrostichum angulatum* Blume, Enum. Pl. Jav. 101, 1828; Fl. Jav., Fil.
t. 6, 1829; Fee, Mem. Fam. Foug. 2: 32, 1845; Kunze in Linnaea 24: 248, 1851; Ch
in Neue Denskschr. Schweiz. Ges. Naturw. 36(1, 2): 46, 49, 1899.

ynonyms: *Acrosticum conforme* sensu Hook., Sp. Fl. 5: 198, 1864. pro parte, n
Swartz; Hook & Bak. Synopsis Fl. 401, 1868.

Acrostichum marginatum sensu Thw., Enum. Pl. Zeyl. 380, 1864; non Wall. ex

Elaphoglossum laurifolium sensu Bedd. Ferns S. Ind. 67, t. 200, 1864; non T. Mo

Elaphoglossum latifolium sensu Bedd. Handb. Ferns. Brit. Ind. 416, 1883; no
Smith

Elaphoglossum krajinae Biswas in Bull. Misc. Inf. Kew 1939: 240, t. fig. 2, 193

hizome long, horizontally creeping, thick, rigid, 4–5 mm dia., densely clot
ith pale, brown, thin walled, papery, ovate scales apex acute, margin en
–5 × 2 mm); Fronds distantly placed in two rows on the dorsal side of the rhizo

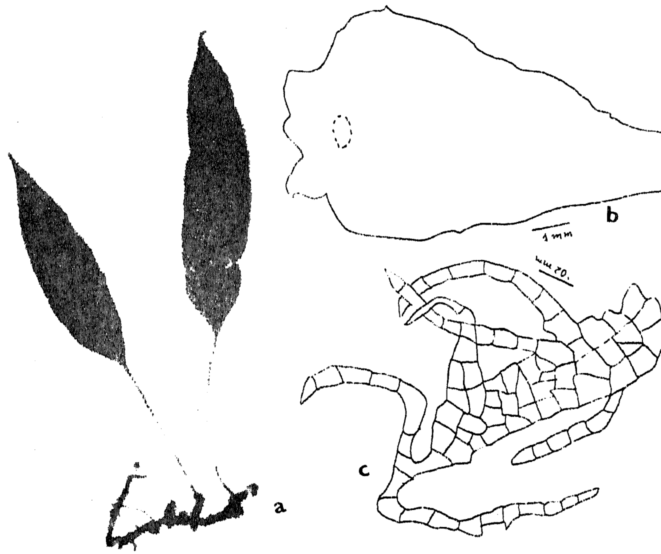


Figure 2. **a.** *Elaphoglossum angulatum*, **b.** Rhizome scale, **c** Lower sur

1913, *Rev. Aug. Sauliers* 1017, 1018 (CAL-25235, 25236); Pulney hills *Beddome* 117, Bearsola, Kodaikanal, ± 2000 m, *S. Chandra & Pari* 6111).

It grows on rocks or on trees as epiphyte.

Distribution: South India, Ceylon, Java, Borneo, Sumatra.

This fern is usually named *Elaphoglossum conforme* or *E. l* *E. latifolium*), (Beddome 1863, 1883, Clarke 1880). Sledge (1967) clearly "they certainly resemble African *E. conforme* but they agree far bett identical with authentic specimens of Blume's *A. angulatum* at Kew a gatherings from Java". It is now established that this Indian fern is neith nor *E. laurifolium* but *E. angulatum*.

3. ***Elaphoglossum commutatum*** (Mett.) Alderw. van Rosenb., M Suppl. 1: 427, 1917; Sledge, Bull. Br. Mus. (Nat. Hist.) 4(2): 90, 196

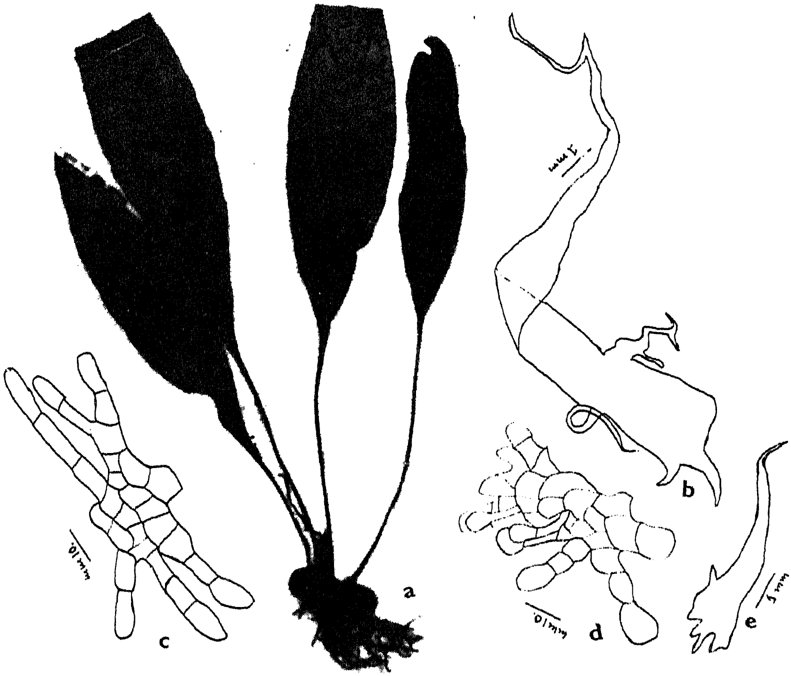


Figure 3. **a.** *Elaphoglossum commutatum*, **b.** Rhizome scale, **c.** Lower surface s
d. Stellate scale of stipe, **e.** Lanceolate scale of stipe.

10 mm long, black, Sterile frond: stipe scaly, scales two types: linear fimbricate, d
own, and with adpressed small stellate scales, but becoming smooth on age. Lam
riaceous, elliptic or narrowly elliptic, 10–20 × 2–2·5 cm, broad, b
adually narrowed and decurrent on stipe, apex acute, margin with narr
rtilaginous border, Lower surface with minute, scattered glandular stellate sca
oper surface glabrous; costa beneath slightly sulcate, upper surface raised. Ve
ce or twice forked, close, ends below margin, hydathodes wanting. Fertile fro
ghtly longer than or equal to the sterile one, stipe is longer, Lamina: Ellip
–20 cm long 3–3·5 cm broad.

ecimen examined: India: Tamilnadu, Pennimadu, Anamalais, Coimbatore D

5–9 mm long, margin entire except for a few teeth at the base, apex ac
two rows on dorsal side of the rhizome, Phyllopodia: short, 4–5 mm lo
ous, densely covered with scale similar to that of the rhizome, deep
frond: stipe 3–10 cm long, brown, scaly, scale similar to that of the rhiz
forming ridge; Lamina coriaceous, linear-lanceolate, 15–35 cm long 1·
apex abruptly narrowed, acute, slightly acuminate, base gradually na
rent up to the base of stipe forming narrow wings or ridges, margin enti
cartilaginous border, reflex on drying; costa: below sulcate, plane, raise
surface with minute brown glandular stellate scale, upper surface ne
veins once or twice forked, prominent, ends below the margin, hyda
Fertile frond: as long as sterile frond but stipe longer, Lamina 1·5–2

Specimen examined: India: Kerala: Darbhakulum Forest, 1000
Trivandrum Dist. Jan. 1., 1974, *N. C. Nair et S. R. Ghosh* 51704 (CAL-
1974 *Nair et Ghosh* 51844 (CAL-6865); Jan. 1974, *Nair et Ghosh* 516
Jan. 5, 1957, *B. K. Nayar* 45072 (LWG); Kuntipura river bank, Silent
Dist. April 21, 1980, *Vohra et Ghosh* 56309 (CAL-7821). Tamilnadu
± 2100 m, Nilgiri, March, 30, 1872, *C. B. Clarke* 11391 (CAL-2524
Lithophyte or epiphyte in deep shaded forest.

Distribution: South India. Endemic.

Elaphoglossum thomsonii S. R. Ghosh et A. Biswas, J. Econ. Tax. Bot. 4(3): 9
83.

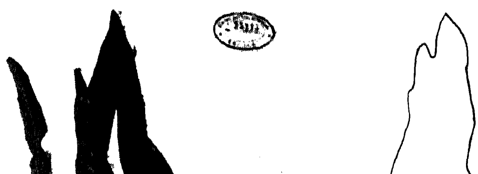
izome creeping, narrow; palea ovato-lanceolate, non attenuate pale brown, ap ute, cells elongate, base peltate, thin, margin with a few teeth. Fronds in several ro t firm. Phyllopodia: present, black, narrow; Sterile frond: stipe 5–10 cm long, da own, furrowed, not firm, scaly, scale lanceolate; Lamina: thin, 15–10 cm long, cm broad, lanceolate, black on drying, base gradually narrowed, decurrent formi ving, apex gradually narrowed, slightly acute, margin thinly cartilaginous, sligh lex. Costa beneath glabrous, slightly raised, flat on upper surface, both surf brous; not firm, vein slightly distinct on the upper surface, not distinct on the lo face, forked once or twice, hydathodes absent; Fertile frond: same size as t the sterile frond, stipe longer than the sterile stipe, twisted, 15–18 cm lo –2·7 cm broad, narrowly lanceolate.

ecimen examined: India: West of Sikkim, Nov. 11, 1857, *T. Thomson* s. n. (c
25245). Type
Epiphyte on trees.

stribution: India—Sikkim, Endemic.

Elaphoglossum cherapunjii S. R. Ghosh et A. Biswas, J. Econ. Tax. Bot. 4(3): 9
83.

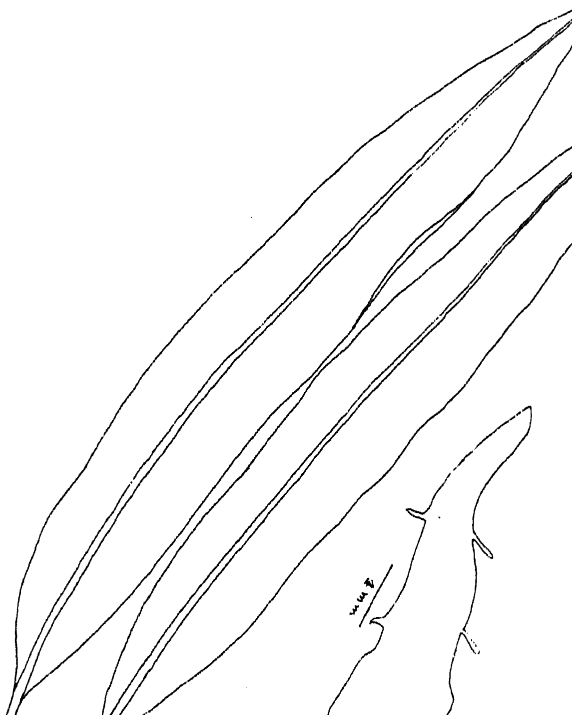
izome short, rigid, suberect, palea dark brown, about 6–7 mm long and 1–0·5 oad, margin and base with a few cilia, apex acute; Fronds in two rows, sciculate. Phyllopodia: 5 mm long, pale black, indistinct; Sterile frond: stipe ri unded, brown, 6–8 cm long, scaly at base, Lamina: coriaceous, wrinkled, brown ying, ovate, 20–25 cm long, 3–2·5 cm broad, base abruptly cuneate and sh



decurrent, apex gradually narrowed and short acuminate. Marg
cartilaginous border; Costa on lower surface flat, and upper surface
Both surface glabrous, veins free, indistinct on both surface, Hyc
Fertile frond not seen.

Specimen examined: India: Meghalaya: Side Cherravalley, belo
±300 m, Dec. 20th 1850, *Dr. J. Faver* s. n. (CAL-25228). Type.

Distribution: Eastern India, Endemic.



Elaphoglossum meeboldii A. Biswas et S. R. Ghosh sp. nov.

ıizome breviter repentia vel suberecta, crassum, rigidum, paleae 8–10 mm lon ; mm lata, atro brunnea lineari-lanceolata, cellulis scleroticis, regulatim pe gularibus, marginibus pilis irregulariter ciliatis, ciliis leviter glandosis; phyllopo evissimus, distinctae, nigrae, 4–5 mm longa, Frondes 3–4, caespitosae; Fr erilis: stipes 2--3 cm longus, squamatus, squamis rhizomatibus non similib formis: lanceolatis et ± stellatis, squama lanceolata ad marginem ciliata, ci ındosis, Lamina: integra, anguste, elliptica, sub-coriacea, 12–15 cm longa, 1–5 a, ad basim et apicum gradatim angustata, ad basin stipitis basi decurrens, arginem cartilaginea et anguste reflexa, costa infra sulcatis, pagina superbusis glab uamulae pagina inferioris minutae, brunneis, laciniato-pectinatis, ciliis lev ındulosis, cellulis poucis globosis, glandulosis prope basin prae-ditae; vanae liber scurae interdum prominentes, simplices vel-1 furcatae, Frons fertiles: non visa Rhizome short creeping, or suberect, thick, rigid; Palea 8–10 mm long and 1·5 r

broad, dark brown, linear lanceolate, with regular pentagonal scleroti
irregularly ciliated, cilia slightly glandular. Phyllopodia very short,
distinct, black. Fronds 3–4, crowded, sterile frond: stipe 2–3 cm long, s
like that of rhizome, two types, stellate as well as lanceolate, margin of la
with glandular cilia. Lamina entire, narrowly elliptic, sub coriaceous, 1
and 1·5 cm broad, base and apex gradually narrowed, base of lamina d
phyllopodia, margin with thin cartilagineous border and slightly re
grooved on lower surface; upper surface of lamina glabrous, lower
minute, brown, ± stellate scales with glandular cilia; base of cilia
glands; veins free, obscure, sometimes prominent, simple, once forked, fe
seen.

Specimen examined: India: Nagaland: Kungla, Nagahills, ± 1500 r
A. Meebold 7408 (CAL-Type).

Distribution: Nagaland (Eastern India).

This species is established on the basis of a single collection made b
from Nagahills, Eastern India, and it is allied to Malayan species
norrishii (Hk.) Bedd. but differs from it in having a short stipe, stellate
margin of rhizome scale with glandular cilia, lamina apex acute, not r
rhizome scale acuminate, not tapering to a point.

8. ***Elaphoglossum indicum*** A. Biswas et S. R. Ghosh sp. nov.

Rhizoma breve repens, rigida, oblique ascendens, densae paleis vestita
ovato-lanceolata, marginibus pilis longis ciliata, apice longe acumina
capilliformis, Fronds polyseriatae ferens, phyllopodia adsunt autem
brunneola, brevissima, 3–4 mm longa, Fronds sterilis: stipes 7–14 cm
neus, paleis eis rhizomatis simililes sed dentibus brevibus praeditae, Lam
longa, 2–3·5 cm lata, apice angustata, basi breviter decurrens, ad margi
cartilaginea, costa infra plana, supra sulcata, textura: subcoriacea,
infra squamulae minutae, sparsae, brunneae laciniato-pectinatae cil
venae obscurae, liberae, simplices vel-semel furcatae, hydathodus
fertiles: non visa.

Holotypus: 27 mile from Shillong, Meghalaya, Sept. 29, 1956, *G. Panigr*


c
1 mm
b

9. *Elaphoglossum marginatum* (Wall. ex Fee) T. Moore, Ind. Fil.: 8, 18

Basinym: *Acrostichum marginatum* Wall. Cat. n. 17, 1829, nom. nud. e Fam. Foug. 2: 31, 1845, excl. syn. Blume.

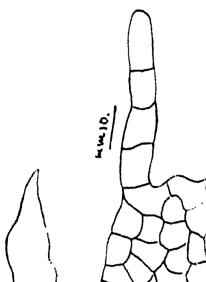
Synonyms: *Acrostichum conforme* sensu Hk., Sp. Fil. 5: 198, 1864 pro Bak. Synop. Fil.: 401, 1868 pro parte.

Elaphoglossum conforme sensu Bedd., Handb. Ferns Brit. Ind. 41 Fig. 247, non J. Smith.

Plant epiphyte, rhizome hard, obliquely ascending, densely cover brown, thick-walled, narrowly lanceolate attenuate peltate scale; margi with very short fimbriation, cells of scale regularly arranged and rectan crowded together in several rows on dorsal side of the rhizome, Phyllopodia distinct, 5–6 mm, stipe jointed, joint not prominent, twisted scaly, scale similar to rhizome scale; Lamina: thick coriaceous, narrow gradually narrowed at both ends, decurrent on stipe, apex acute: Margi cartilaginous border, strongly reflex; Midrib raised on abaxial surface adaxial surface, upper surface glabrous, lower surface with scattered r stellate scale with cilia, cilia with glandular apex; veins obscure, once or ends 1 mm below the margin, hydathodes lacking. Fertile frond: stipe lo of sterile one; lamina contracted.

Specimen examined: Nepal: Sheopore, 1829, *Wallich* 17 (Type, CAL-2522 s. n. (CAL).

India: Meghalay: Elephanta falls, ± 1600 m upper shillong *B. K.* (LWG-57725); same locality, *P. K. Hazra* 25577 (ASSAM); Lyndoh fo



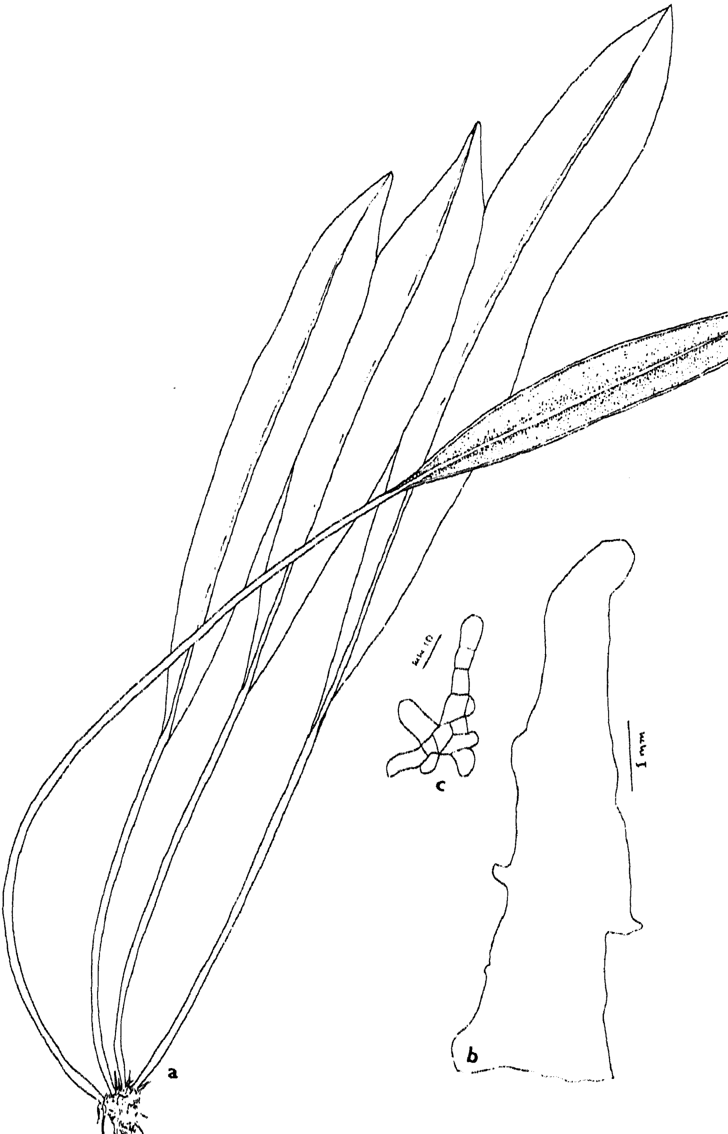
m, Sept. 8, 1913, *U. Kanjilal* 2505 (ASSAM). Nagaland: Pulim Bazar, Sept.
). *Prain* s. n. (CAL).
tes or lithophytes.

on: Nepal, Eastern Himalayas.
chum marginatum Wall. Cat. n. 17 was validly established by Fee (1845); (1864, l.c.), Beddome (1863) and Clarke (1880) confounded it with *ssum conforme* Sw. Beddome (1883) omitted this species, Sledge (1967) that under cartilaginous leaved species only this taxon occurred in the s. But our study shows that a few others new species also exist in the imalayas. This species differs from all others by blackish, attenuate rhizome wded stipes, lamina margin strongly reflexed, and surface wrinkle, fertile ch longer than the sterile one.

hoglossum fasciculatum A. Biswas et S. R. Ghosh sp. nov.

a breviter repentia, crassum, rigidus, obliqua ascendens; rhizomatis palea ongae, 1–1·5 mm latae, bruneae tenuiter, peltatus, nonacuminatae, ovato-s, marginibus leviter fimbriatus; Phyllopodia 6–8 mm longa, adsunt autem ua, tenuiter brunea, Frondes polyseriatus ferens, fasciculates; Frons sterilis: 0 cm longa, stramineus, paleis eis rhizomatis similibus; Lamina simplices, longae, 2·5 to 4 cm latae, integrae, rugusus, ellipticus, coriacious, decur-apice obtusa; costa subtus squamatous, utrinque planus, in sicco pallede .amina dorso squamulae minutae, sparsae, bruneae tenuiter, plus minus apicem cellulis glandulosi, Venae obscurus sed vix prominentes, simplices ati, Hydathodes nulli. Frons fertilis: Breviter, stipes 12–15 cm longa, lamina gae, 1·5–2 cm latae, basi abrupte angustata apice late obtusa.
ne short creeping, stiff, thick, obliquely ascending, densely covered with light –4 mm long, 1–1·5 mm broad, peltate, ovate-lanceolate scale; apex not ed, margin with short teeth; Phyllopodia 6–8 cm long, inconspicuous, pale ronds crowded together in several rows on the dorsal side of the rhizome, us; Sterile fronds: stipe 6–10 cm long, stramineous, palea similar to , Lamina coriaceous, 15–30 cm long, 2·5 cm broad, wrinkled, elliptic, base , apex obtuse, costa scaly, raised on the lower surface, flat on the basal part of


a
b
c
2 mm


a
b
c
1 mm

Holotype: Kalapani hills, Meghalay, Eastern Himalayas; *Simons* s. n

Distribution: Meghalaya, Eastern Himalayas.

This species differs from *Elaphoglossum marginatum* by stipe non tw rachis reddish, lamina obovate, phyllopodia distinct.

12. ***Elaphoglossum himalaycum*** K. P. Biswas ex A. Biswas et S. R.
E. himalaycum K. P. Biswas MSS.

Rhizoma breviter repentia, regidus: paleis dense vestita, bruneae, ter longa et 2–2·5 mm latae, tenuis, anguste ovatus, peltatus, irregulariter Phyllopodia, 2–3 mm longae, adsent outem insconspicua, bruneae; Frons steriles: Stipes 9–10 cm longa, bruneae, palieis rhizomatis sin simplices, integrae, ellipticus, 20–22 cm longa et 3·5–4 cm latae, basin angustata, besi breviter decurrens, marginibus cartilagineous, costa i planae, costa supra penitus sulcatis; Texturae: coriaceous, in sicco b supra sparsae paleaceae, et glabra, infra squamulae minutae, brune apicum cellulis glandulosis, cellulis paucis globosis glandulosis prope b Venae obscurus, simplices, vel 1 furcati, 1 mm inter se distantes; Hy Frons fertiles: stipes longiore, 13–18 cm longae, tortae, Lamina: elli apice abrupte angustatus, apice acuta.

Rhizome short creeping, rigid, densely covered with light brown ovate, paltate scale, scale 7–8 mm long and 2–2·5 mm broad, margin Phyllopodia: 2–3 mm long, inconspicuous, brownish; Frond: 2–3, stipe: 9–10 cm long, brown, scale similar to rhizome scale; Lamina: co 20–22 cm long, 3·5–4 cm broad, base and apex abruptly narrow decurrent, margin with cartilagineous border, costa beneath scaly, p adaxial surface; upper surface almost glabrous, Lower surface with mi stellate scale, apex of cilia glandular, glands also present at the base obscure, simple, once forked, ends 1 mm below the margin, Hydat Fertile fronds: stipes longer, 13–18 cm long, twisted; Lamina: elliptic abruptly narrowed, apex acute.

Holotype: Sikkim, ± 2100 m, *Levinge* s. n. (CAL).

Distribution: India: Sikkim Himalayas.


c

Figure 13. **a.** *Elaphoglossum prainii*, **b.** Rhizome scale, **c.** Stipe scal

surface glabrous, lower surface with sparsely brown, adpressed, ± ste
free, distinct on the lower surface, indistinct on the upper surface; Fert
and stipe longer than the sterile one.

Specimen examined: India: Palanbazar, Nagahills, Sept. 1886, *Dr*
(CAL-25233) Type.

Distribution: Nagalands, Eastern India

argo tenuiter cartilagineous, costa subtus leviter prominens, sulcatis, Textur riaceous, pagina superioris glabra, pagina inferioris squamulatus, squamu nuta, ± stellati, apicem cellulis glandulosis, cellulis poucis globosis glandulo ope basin praeditae; Venae obscurus, 1-furcati, hydathodes nulli. Frondes fertili pitis quam frondes sterilis longiore.

Plant small, rhizome, short, suberect, covered with linear lanceolate, yellowish sca ex nearly acute, margin with a few glandular cilia; Phyllopodia: present, ste ond: stipes not firm, 2–7 cm long, stramineous, sulcate, palea similar to that izome scale; Lamina coriaceous, simple, entire, narrowly lanceolate, apex s otuse, base longe decurrens, Margin with thin cartilaginous border, costa bene ghtly prominent, sulcate; upper surface glabrous, lower surface with minute bro stellate scale with cilia, apex of which glandular, base of cilia provided with globb ands; Veins obscure 1 furcate, hydathodes absent, Fertile frond longer than erile one.

olotype: Lacheng ± 2700 m, Sikkim, Aug. 9, 1892, *Gammi* 701 (CAL).
aratype: Sikkim, Sept. 1882, King's collector (CAL).
ther specimen examined: India: Meghalaya: On the way to Mouflong, Cherapu Sept. 25, 1926, *J. C. Banerjee* s. n. (CAL).
Epiphytes.

istribution: India: Meghalaya, Sikkim.

On the basis of his own collection from the above Shaila, Khasia, Meghalaya, Cla 880) expressed that his collected specimens were identical with *Acrosticum g*

goneum Kaulf. From the study of Anderson and Crosby it
Elaphoglossum gorgoneum (Kaulf.) Brack. is endemic to the Hav
moreover its veins does not unite at the margin. Most probably Clar
new species *Elaphoglossum sikkimense*, although we have not seen th
at the CAL herbarium. All other characters mentioned by Clarke
species.

15. ***Elaphoglossum nilgiricum*** Krajina ex Sledge, Bull. Brit. Mus
4(2): 94, 1967; Nayar et Kaur, companion to Bedd. Handb. ferr
1974.
Elaphoglossum squamosum sensu Bedd. Ferns S. India, 67, t. 1
Ferns Brit. Ind. 420, t. 251, 1883 excl. specimen ex Ceylon non

Rhizome short, obliquely ascending or even upright densely cover
lanceolate, or linear brown scale, apex acute, margin with black te
indistinct. Fronds crowded together and inserted all round the rhizo
long, covered with wooly lanceolate scales, margin of the scales p
concolorous setiferous teeth. Lamina: simple, 8–25 cm long, obl
cuneate or attenuate, apex abruptly narrowed, sub-obtuse to obtu
upper surface green to brown, lower surface green; upper surface sp
surface hidden under the dense coating of pale brown wooly scal
surface vary in shape, provided with narrow to broadly lanceolate
orbicular with long setiferous teeth.

Specimen examined: India: Tamilnadu: Sispara Ghat, ± 1800 m, N
13468 (CAL); June, 1859, *Beddome* s. n. (CAL). Kerala: Kuntipuj
dam, Silent Valley, Palghat Dist. *R. K. Ghosh* 56341 (CAL).
Epiphytes.

Distribution: South India, Ceylon.

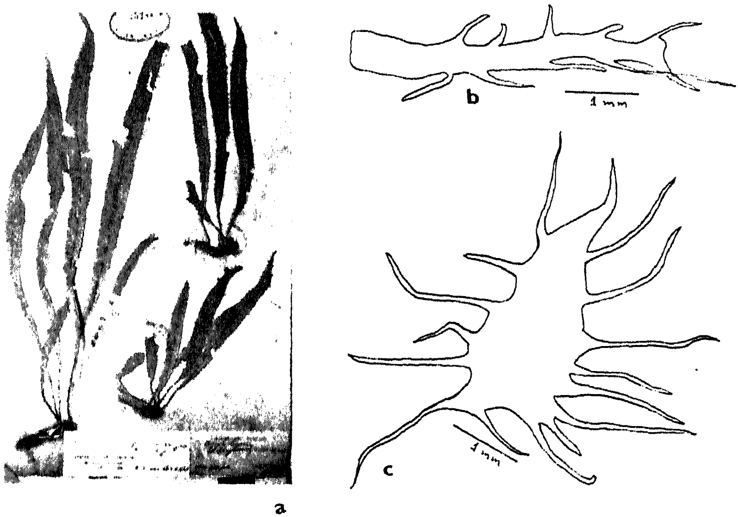


Figure 16. **a.** *Elaphoglossum ceylanicum*, **b.** Rhizome scale, c. Lower surface scale.

. ***Elaphoglossum ceylanicum*** Krajina ex Sledge, Bull. Brit. Mus. (Nat. Hist.) B 4(2): 95, 1967; A. Biswas, J. Bom. Nat. Hist. Soc. 80(1): 267–68, 1983.

nizome creeping, thick, clothed with linear, dark chesnut or almost black scales, sc argin with black concolorous setiferous teeth. Phyllopodia: indistinct, Fronds tuft pe 1·5–5 cm long, scaly, scale narrowly lanceolate, light brown, membraneo argin with dark or almost black setiferous teeth. Lamina: simple, 8–25 cm lo -2 cm broad, oblong elliptic, base attenuate, apex acute to shortly acumina xture: thin, lower surface dull green, both the surfaces scaly, but neither surf vered by dense coating of scales, scales of both surface vary in shape, broadly rrowly ovate or sometimes orbicular, margin of which fringed by long setifer eth. Fertile frond similar to sterile frond.

ecimen examined: India: Sikkim, ± 1200 m, April 13, 1857, *Thomson* s.n. (CA 288).

rlier record: Ceylon.

Epiphytes.

cartilaginous, costa beneath rounded, prominent; scales on upper s
adpressed to the surface, centre broad, pale, scales on lower surface r
stellate, adpressed to the surface, on older fronds scale soon abraded; V
lower surface, ends in indistinct slightly swollen hydathodes. Fertile fro
sterile, 20–25 cm long 1·5 cm broad, linear.

Specimens examined: India: Assam: Sairang, Nov. 26, 1953, *B.* (CAL-25227); Jowaipungi, ± 1500 m, *G. Mann.* s.n. (CAL-25248); Jov Aug. 1892. *Dr. King's collector* s.n. (CAL-25226) Type. Nagaland: Jaih March 1, 1882, *H. Collett* 73 (CAL). Sikkim: Gangtok ± 1000 m, Jur 304 (CAL). Nepal: Nepalia, *Wall* Cat. n. 16 (Microfisches) Tundrija 1950, *K. S. Srinivasan* s. n. (CAL-44354). Bhutan: E. Bhutan, Nov. *Balkrishnan* 44676 (ASSAM).

Distribution: Nepal, Bhutan, E. Himalayas.

18. ***Elaphoglossum blumeanum*** (Fee) J. Sm. Ferns Brit. & For. 106, Flora Males. Ser. 2, 2(4): 311, 1978.

Basinym: *Acrostichum blumeanum* Fee, Hist. Acrost. 62, 1845, excl *blumeana* Presl.

Synonyms: *Acrostichum viscosum* (non Sw.) Bl. Fl. Jav. Fil. 27, 1829.
E. petiolatum [(non Sw.) Urb.] v. A. v. R. Handb. 717, 1908.
Elaphoglossum yunnanse [non (Baker) C. Chr.] Holttum Rev. Fl. Ma 1954.

iizome creeping, bearing fronds in two ranks, scales 4–5 mm long, dark brow
ossy, rigid, linear, bearing a few teeth. Phyllopodia: 6–8 mm long. Sterile frond: sti
–12 cm long, at base with scales as rhizome but with many stiff marginal hairs, a
rsistently covered with stellate scale; Lamina 35–40 cm × 2–3 cm, thin, ba
rrowly cuneate, apex acuminate, margin not cartilaginous, costa beneath rounde
ominent on lower surface, scales on upper surface thin, pale, appressed with lo
nder marginal hairs, scales on lower surface small, with 3–5 stiff marginal ha
reading away from the surface, on old frond scales soon abraded but leaving re
ts at the point of attachment. Fertile frond: stipe 20–25 cm long.

ecimen examined: Khasia, ± 1200–1500 m, Meghalaya, *Hooker et Thomson* s.
(CAL-25276).
ew record for India.

istribution: India: Eastern Himalayas, Malaysia: Sumatra, Java, Borneo, Celeb
Phillipines.

. ***Elaphoglossum stelligerum*** (Wall. ex Bak.) Biswas et Ghosh Com. nov. Sled
Bull. Brit. Mus. Nat. Hist. 4(2): 92, 1967, excl syn. of *Acrostichum neriifolium* Wa
Cat. n. 16, 1829. but not other synonyms
sinym: *Acrostichum stelligerum* Wall. Cat. n. 2167, 1829 nomen nudum.
Acrostichum stelligerum Wall. ex Bak. in Hook. & Bak. Synop. Fil. ed. 2: 521, 18
Elaphoglossum stelligerum T. Moore Index Fil.: 368, 1862, nomen nud:
Acrostichum viscosum sensu Hook., Sp. Fil. 5: 220, 1864 pro parte, non Swar
Bedd. Fern. S. Ind.: 67, t. 196, 1864; Handb. Ferns. Brit. Ind. 420, 1883; pro par
non J. Smith.

hizome creeping, bearing fronds in two ranks, scales 4–5 mm long, dark brow
-5 mm long, bearing a few teeth or stiff hairs at the lower parts; Phyllopodia 8–10 m
ng, blackish, sterile frond: stipe 5–15 cm long, covered with lanceolate scale w
any stiff hairs at the margin and together with stellate brown scale; Lami
nceolate, 10–30 cm long, 2–2·5 cm broad, apex acuminate, base narrowed but
current on the stipe, margin not cartilaginous, costa beneath rounded, promine
ales on upper surface stellate with long arm and broad centre, adpressed, thin, pa
ales on the lower surface loose, rufous brown, stellate with 6–10 long arm, cen

Figure 18. **a.** *Elaphoglossum stelligerum*, **b.** Rhizome scale, **c.** Lance
d. Stellate scale of stipe, **e.** Stellate scale of upper surface, **f.** Lower su

On the basis of *Wight's* collection from Nilgiris, S. India, *Dr. Wal*
proposed the name *Acrostichum stelligerum*. *Baker* (1874, l. c.) provid
of this species under *Acrostichum*. *Moore* (1862, l. c.) in his index
species under the genus *Elaphoglossum* without giving any descriptio
Sledge (1967) has not properly transferred this species under *Elaphoglo*
new combination is proposed here.

20. ***Elaphoglossum khasianum*** A. Biswas et S. R. Ghosh sp. nov.

Rhizoma repens, frondes biseriatus ferens; palea lineari-lanceolate,
marginibus poucis ornatae, apice non acuminatae; Phyllopodia 2–3 n
2–10 cm longa, squamatus, squmulae biformis, lanceolatis et ± st

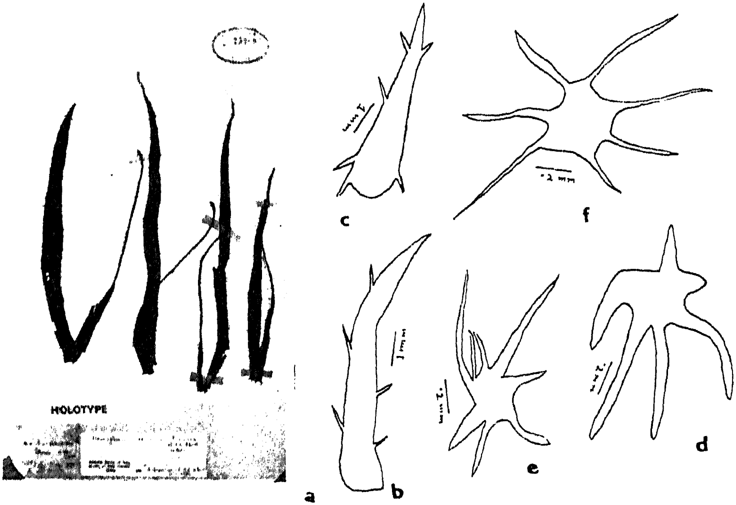


Figure 19. **a.** *Elaphoglossum khasianum*, **b.** Rhizome scale, **c.** Lanceolate scale **d.** Stellate scale of stipe, **e.** Lower surface scale, **f.** Upper surface scale.

Rhizome creeping bearing frond in two rows, scale linear-lanceolate, brown, with few teeth, apex not acuminate, Phyllopodia 2–3 mm long, sterile frond 2–10 cm long, scaly, scale two types, lanceolate and ± stellate, margin of lan scale with a few teeth; Lamina: linear, 5–25 cm long 0·4–1·5 cm broad, apex gr long acuminate, base gradually narrowed, margin strongly reflex on drying below prominent, veins not distinct on lower surface, upper surface scal adpressed, stellate; Lower surface scale brown, stellate with 5–7 margina hydathodes wanting, Fertile frond: Stipe 10–15 cm long, Lamina 15–16 cm lon broad, apex gradually narrowed, base narrowly cuneate, apex non acuminat

Distribution: Eastern India: Meghalay, Arunachal Pradesh.

Abbreviations: ASSAM—Herbarium, Botanical Survey of India, Shillong. CAL— National Herbarium, Botanical Survey of India, Howrah (Calcutta).
LWG—Herbarium, National Botanical Research Institute, Lucknow.

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Typology and taxonomic value of foliar sclereids in the Proteaceae II. *Adenanthos* Labill.

T ANANDA RAO and SWAPNA CHAKRABORTI
Botany Department, Bangalore University, Bangalore 560 056, India

MS received 14 June 1984; revised 24 August 1984

Abstract. A majority of *Adenanthos* species possess diffuse sclereids. They are classified i major types. Their presence or absence is listed in Nelson's revised scheme of classification this genus to indicate the overall perspective of their use in taxonomy. Further taxono problems alluded to by Nelson are illuminated here by sclereid typology.

Keywords. Foliar sclereids; typology; *Adenanthos*; Proteaceae.

Introduction

is genus is endemic to Southern Australia especially in parts with a mediterranea pe climate (Aschamann 1973). The main concentration of species is in South-W d Western Australia, South Australia and Victoria. Recently *Adenanthos* w vised taxonomically and a new section has been established (Nelson 1978).

Notwithstanding the reported occurrence of sclereids in a few species of this gen ere is no detailed study on their typology and taxonomic implications. In ma stances the accounts are inadequate for taxonomic judgement (Jonsson 18 lereder 1908; Metcalfe and Chalk 1950; Rao and Bhattacharya 1978; Rao and D 79).

Materials and methods

e materials for this study were procured from the following herbari urators/Directors. These herbaria are listed, following the symbols published olmgren *et al* (1981).

Adenanthos acanthophylla George, Western Australia, Hamelin Port to Tame

Meisn., W. Australia, N. W. of Bedshingarra, A. S. George 6723 (PERT
George, W. Australia, West Barran range, E. Croxford *s.n.* (DBN);
K. Newbey 1451 (PERTH). *A. eyrei* Nelson, W. Australia, E. C. Nelson AN
(type material). *A. filifolia* Benth., W. Australia, Max Koch 1767 (CAL);
Bluff Knott, Stirling range, R. D. Royce 6053 (PERTH). *A. flavidiflo*
W. Australia, slope of East Mount, Barron and Croxford *s.n.* (CAL, DBN);
slope of E. Mount, Barren and Croxford *s.n.* (DBN); W. Australia, nea
Rocks, R. A. Jaffery 430 (PERTH). *A. forrestii* F. Muell. W. Australia,
A. S. Weston 8274 (PERTH). *A. glabrescens* Nelson, subsp. *glabrescens*
Australia, 10 miles SE of Lake King, A. S. George 3653 (PERTH). *A. grac*
W. Australia, 6 km or on peak Charles, A. S. George 15106 (PERTH). *A. i*
W. Australia, near Red Lake, E. C. Nelson ANU 17007 (DBN). *A. labillar*
West Australia, Higher slopes of Whoogarup range, A. S. George
A. linearis Meisn., W. Australia, Stirling range, E. Wittwer 600 (PERT
podiana Nelson, Bayswater, Lower Swan River, Alex Morrison *s.n*
meisneri Lehm., W. Australia, Dt. Wellington, E. Pritzel 98 (CAL). *A. ob*
W. Australia, South-West, E. Pritzel 246 (CAL); W. Australia, Kirrup.,
(CAL). *A. oreophila* Nelson, East Mt, Barren, G. A. Gardener *s.n.* (PERT
Meisn., W. Australia Ongarup, K. Newbey 917 (PERTH). *A serice*
Australia, South-West, E. Pritzel 245 (CAL). *A. sericea* Labill., subsp. s
W. Australia, Albany, P. Sanderson *s.n.* (DBN). *A. sericea*, subsp. *sphaln*
Australia, Thistle cave, Cape le Grand National Park, E. C. Nelson ANU
A. sticta A. S. George, W. Australia, C. A. Gardner *s.n.* (PERTH). *A. ter*
W. Australia, Lofty range, B. Kaspiew 147 (CAL). *A. venosa* Meisn., Ba
Croxford *s.n.* (DBN); W. Australia, Mount Barren, A. S. George 6976 (PE
Hameraley R. estuary, A. S. George 7219 (PERTH). *A. cuneata* × *dobsoni*
17071 (DBN). *A. cuneata* × *forrestii* Nelson, ANU 16981 (DBN). *A. ma*
terminalis Nelson, ANU 17038 (DBN).

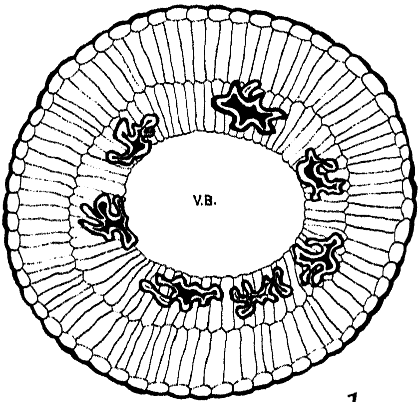
The clearing technique is after Rao and Naidu (1981), and the sclerei
after Rao and Bhupal (1973).

3. Observations

Studies on cleared leaves, transverse sections and macerations of
Adenanthos revealed that sclereids of different forms are present in

A. drummondii Meisn. (A. S. George 6723, PERTH) (figures 2, 3), *A. flavidif*
(R. A. Jaffrey 430, PERTH), *A. forrestii* F. Muell. (A. S. Weston
A. labillardierei Nelson (A. S. George 1941, PERTH), *A. linearis* Meisn. (E.
PERTH), *A. macropodiana* Nelson (Alex Morrison *s. n.*, PERTH), *A. ore*
(C. A. Gardner *s. n.*, PERTH), and *A. venosa* Meisn. (A. S. George 697

Sclereids vary from sub-spheroidal to gnarlyform in *A. cygnorum*
cygnorum, *A. dobsonii* and *A. flavidiflora*. In a majority of cases, the sc



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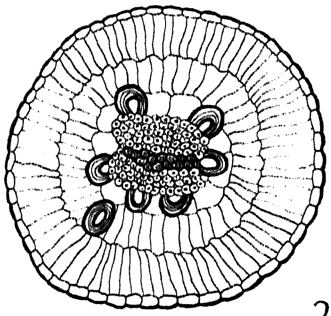

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smooth outline, but in *A. linearis* and *A. venosa* they show varied lobe
resembling vesiculose sclereids (figure 8).

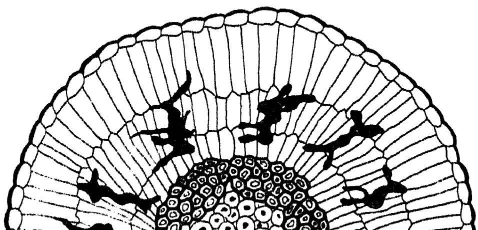
3.1b *Gnarlyform sclereids*: They are thick-walled mis-shapen small-
with protuberences of irregular shapes and size. They have striated cell
occluded lumina and are found scattered in different parts of the meso
observed in *A. detmoldii* F. Muell. (R. D. Royce 3235, PERTH), *A. do*
(A. S. George 7386, PERTH), *A. filifolia* Benth. (R. D. Royce 6053, P
A. meisneri Lehm. (Pritzel 98, CAL), *A. sericea* Labill. (Pritzel 24
terminelia R. Br. (Kaspiew 147, CAL).

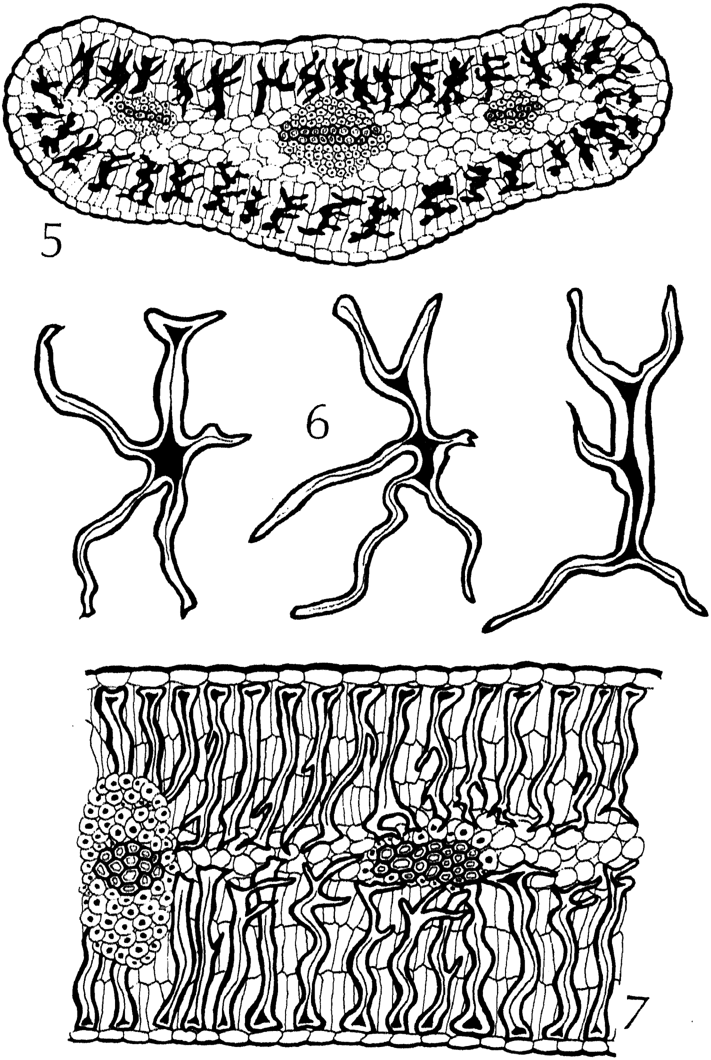
3.1c *Rhizosclereids*: Rooting columnar cell forms with spicule-like
end found in the mesophyll of *A. acanthophylla* George (Nelson 170
elliptica George (K. Newbey 1451, PERTH; figures 12, 7).

3.1d *Palosclereids*: Small-sized palisade-like cell forms with spiculat
observed in *A. glabrescens* Nelson (A. S. George 3653, PERTH; figure

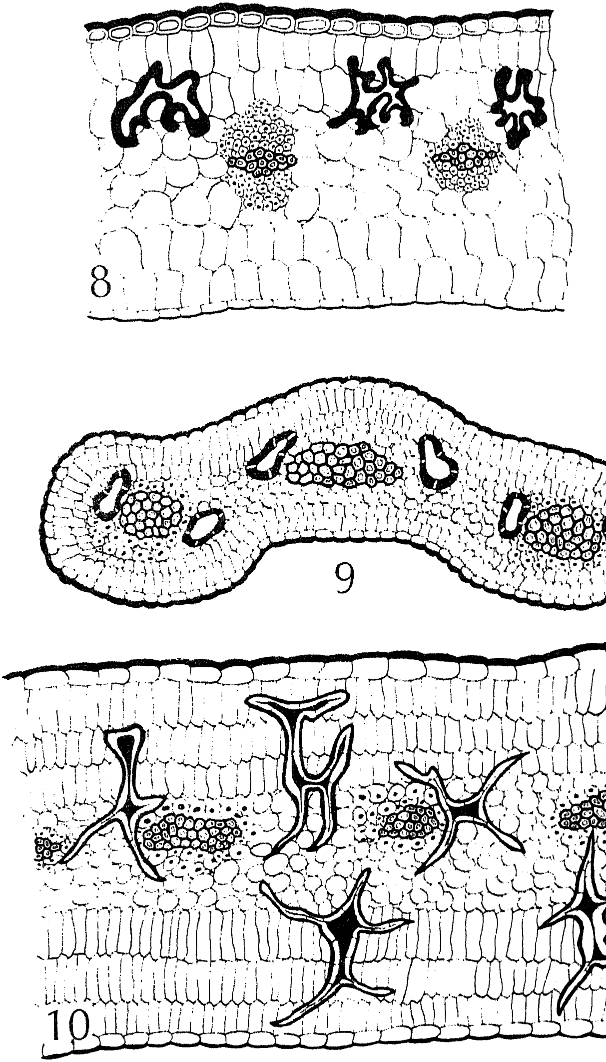
3.1e *Fusiform sclereids*: These sclereids have a broad mid-portion w
ends. Often they are found in rows, and are conspicuous in the mesoph
is exemplified in *A. cunninghamai* Meisn. (Kaspiew 71, CAL).

3.1f *Ramiform sclereids*: These sclereids are more or less I-shaped
attenuated branches at the ends. They are exemplified in *A. cuneata* La
CAL), *A. elliptica* A. S. George (K. Newbey 1451, PERTH), *A. eyrei* Nels
17044, DBN), *A. gracilepis* A. S. George (A. S. George 15106, PERTH; fig
Nelson (Nelson ANU 17007, DBN), *A. pungens* Meisn. (Newbey 9



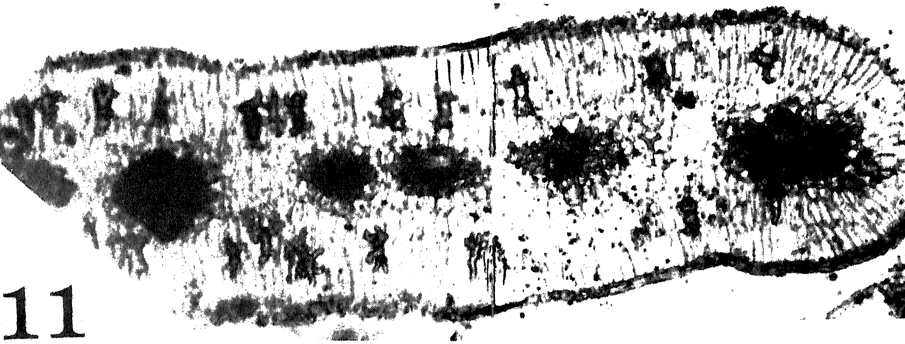


Figures 5–7. TS of the leaf sectors: **5.** *A. cacomorpha* Nelson (K. Newbey 2779, PER
Polyramous sclereids disposed mostly in the palisade layer (× 450). **6.** A few polyram



Figures 8–10. (× 450) TS of the leaf sectors: **8.** *A. venosa* Meis

PERTH); Vesiculose sclereids in the adaxial palisade region. **9.** *A. fo*



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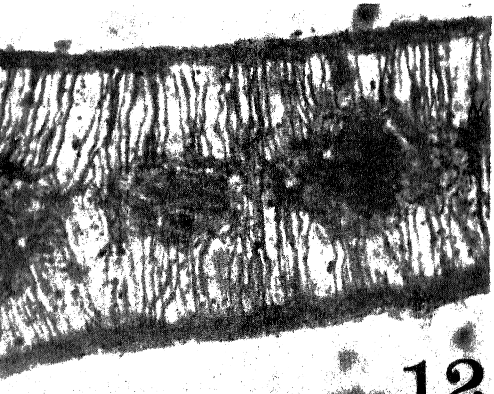

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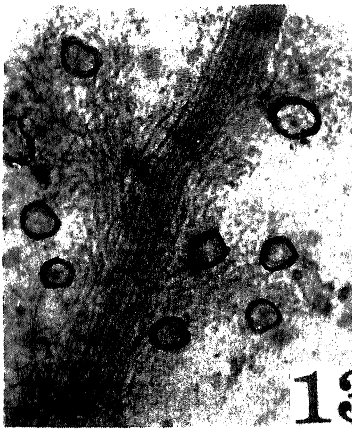

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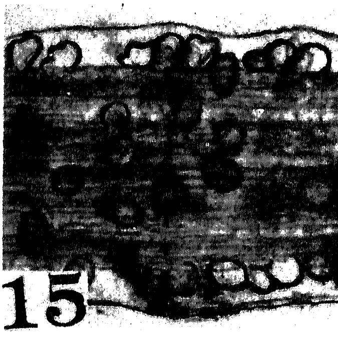

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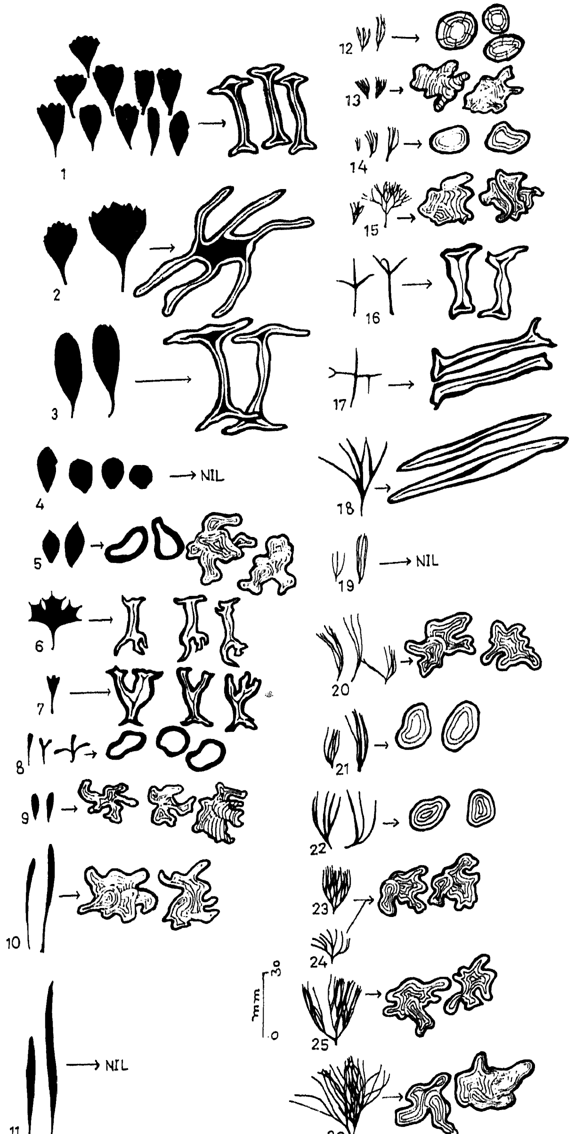
1
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Systematic applicability

ne earliest treatment on *Adenanthos* is that of Bentham (1870). In this conc count, he has recognised two sections, namely, *Eurylaema* and *Stenolaema*. Und is framework, he recognised 14 species. Since then no attempt was made to revise t nus taxonomically, despite the descriptions of a few more species. Recently, Nels 978) based on field population studies has published a useful taxonomical revision is genus. In this account he has recognised two sections: *Eurylaema* corresponding *urylaema* of Bentham and *Adenanthos* with its two sub-sections *Anaclastos* a *denanthos* corresponding to *Stenolaema* of Bentham.

Following Nelson's classification of this genus, an attempt has been made to sort c e varied types of sclereids as discovered in the present work to see how far th esence or absence is helpful in understanding their grouping under different sectio

axa	Typology of sclereids
ction 1: *Eurylaema*	
. *A. detmoldii* F. Muell.	Gnarlyform—infrequently present.
. *A. barbigera* Lindl.	Devoid of sclereids
. *A. obovata* Labill.	—do—
ction 2: *Adenanthos*	
ıb-Sect. *Anaclastos*	
. *A. drummondii* Meisn. (figures 2, 3)	Spheroidal
. *A. dobagii* Nelson	Polyramous
. *A. apiculata* R. Br.	Devoid of sclereids
ıb-Sect. *Adenanthos*	
. *A. linearis* Meisn.	Spheroidal
. *A. venosa* Meisn. (figure 8)	Vesiculose
. *A. elliptica* George (figures 7, 12)	Rhizoform
. *A. glabrescens* Nelson (figure 11)	Paloform
. *A. dobsonii* F. Muell.	Gnarlyform
. *A. pungena* Meisn.	Ramiform
. *A. gracilipes* George (figure 4)	Ramiform
. *A. cunninghamii* Meisn.	Fusiform
. *A. eyrei* Nelson	Ramiform
. *A. forrestii* F. Muell. (figures 9, 13)	Sub-spheroidal

In the above table one could observe the clear morphological occurrence of varied sclereid types. In the first trend the mesophyll of devoid of sclereids. In the next trend the mesophyll possesses diff sclereids. The next trend is the presence of gnarlyform sclereids. Thes shapen sclereids are very conspicuous in the mesophyll. Lastly, t fusiform, ramiform or polyramous types have been encountered.

6. Taxonomic implications

Taxonomic problems alluded to by Nelson (1978) are illuminated l typology.

Among the three species, namely, *A. detmoldii*, *A. barbigera* and *A* Section 1 *Eurylaema*, sclereids are observed only in *A. detmoldii*, wher two species the mesophyll is devoid of sclereids. Nelson (1978 *A. detmoldii* from other members of this section, namely *A. barbigera* a the presence of yellowish orange perianth, tuberculate foliar glands juvenile foliage. The present study reveals that gnarlyform sclereids als diagnostic character to distinguish this species. Further, Nelson's *A. barbigera* and *A. obovata* are closely related and similar is further s the absence of sclereids in the mesophyll of both the species.

Among the three species, namely *A. drummondii*, *A. dobagii* ar included under the sub-section *Anaclastos* of §2 *Adenanthos*, spheroida been recorded only in *A. drummondii*. Thus, the presence of spheroidal vicinity of midrib coupled with other characters like shape of the pe style, tufts of hairs at the throat, as pointed out by Nelson (1978 p. 3 *A. drummondii* Meisn. is a distinct species.

Further, Nelson is of the opinion that *A. dobagii* is similar in ap closely related *A. apiculata* as well as to *A. flavidiflora*. The present stu that *A. apiculata* is devoid of sclereids while *A. flavidiflora* has sub-sphe and *A. dobagii* has polyramous sclereids. These features are sufficient they are distinct species.

Bentham (1870) considered *A. drummondii* to be a synonym o However, Nelson (1978) reported that they are quite distinct morp geographically. This viewpoint is further supported by the recorde spheroidal sclereids in *A. drummondii* and their absence in *A. apicul*

aracters such as flabellate leaves and polyramous sclereids of *A. sticta*, elliptic lea
d ramiform sclereids in *A. elliptica* and flagellate or rarely elliptical tomentose lea
d ramiform sclereids of *A. cuneata* could be considered of sufficient diagnostic val
In *A. glabrescens* although leaves are similar in appearance to *A. linearis* t
lereidal differences in these species outweigh their similarities. In *A. glabrasce*
lereids are paloform whereas in *A. linearis* they are spheroidal. The presence
ssimilar sclereids, does not support Nelson's (1978) contention that *A. glabrescen*
osely related to *A. dobsonii*.

George (1974) considered *A. gracilipes* to be related to *A. apiculata*. Howev
elson (1978) opined that the two species differ in many aspects, most significan
the size and morphology of the perianth. Similarly they differ from each other
esence and absence of sclereids in the mesophyll.

A. pungens and *A. gracilipes* have densely sclereified leaves and also similar ramifo
lereids. *A. meisneri* is distinguished from all other species except *A. venosa*. Howev
e presence of gnarlyform sclereids in both the species does not facilitate th
entification.

For a long time, *A. sericea* and *A. cygnorum* have been confused and some work
garded *A. cygnorum* as a variety of *A. sericea* (Ewart 1907). However, the two spec
ffer not only in their morphological details (Nelson 1978) but also in havi
ssimilar types of sclereids: gnarlyform in *A. sericea* and sub-spheroidal
cygnorum.

A. argyrae is related to *A. flavidiflora* but it is distinguished by the very small usua
isegmented leaves (Nelson 1978). Both the species, however, have similar types
heroidal sclereids.

A. oreophila was earlier considered to be a form of *A. sericea*. However, they
stinct, especially in leaf morphology and anatomy, though the flowers of the two ta
e almost identical. Further, the varied types of sclereids, namely sub-spheroidal
oreophila and gnarlyform in *A. sericea* are distinct features of diagnostic interest
ese taxa.

A. terminalis is distinguished from *A. macropodiana* in having less divided leaves
aller perianth that is cream-green in colour and by the villous style (Nelson 1978)
observed that *A. terminalia* possesses gnarlyform sclereids while in *A. macropodia*
ey are sub-spheroidal in the vicinity of midrib.

A. filifolia is closely related to *A. labillardierei* from which it may be distinguished
e colour of the perianth, the shape of the perianth limb, and the shape of the bra
Jelson 1978). Further, the above taxa can be distinguished by different types

of taxonomic value at the sectional or genus level. On the other han varied types of sclereids is a helpful adjunct in establishing the ident Parallel instances are found in many investigated sclereid bearin flowering plants (Rao 1980).

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iumerical chemotaxonomy of *Bauhinia*

G NAGESHWAR, M RADHAKRISHNAIAH and L L NARAYAN

Department of Botany, Nizam College, Osmania University, Hyderabad 500 001, India
* Department of Botany, Kakatiya University, Vidyaranyapuri, Warangal 506 009, Ind

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Abstract. The chemotaxonomy of twelve species of *Bauhinia* is presented. From quantified data on the distribution pattern of different chemical constituents, including free aminoacids, it is inferred that the taxa are closely related and do not warrant splittin the genus. However, the division of the genus based on certain selected phenolic constitue does not conform to the one on morphological grounds.

Keywords. *Bauhinia*; secondary metabolites; free amino acids; affinity; splitting.

Introduction

he chemotaxonomy of *Bauhinia* received very little attention in the multi-volumino ork on the chemotaxonomy of flowering plants in general (Gibbs 1974) and of guminous taxa (Harborne *et al* 1971) in particular. The present account deals w e distribution pattern of different secondary metabolites and free amino acids a timates the extent of kinship among them and the desirability of splitting the gen

Material and methods

he twelve species of *Bauhinia* presented in table 1 are either procured from t dian Botanic Garden, Howrah or from various localities in Hyderabad. To dete fferent secondary metabolites, fresh leaves and stems and 80 % ethanolic extracts e shade-dried material were used. Following standard procedures/specific te: ibbs 1974), the material was screened for alkaloids, anthraquinones, aucut mpounds (Ehrlich test), aurones (Aurone test A), catechol-tannins (HCl/methar Isenberg Buchnann's test), cyanogenic glycosides (HCN test A), ellagic ac vonoid pigments (Shinoda test), indoles, Juglone (Juglone test A), leucoa

Table 1. List of taxa studied.

	Name of the taxon	Place of collection
1	*B. scandens* L var *horsfieldii* (Miq.) Ohashi (= *B. anguina* Roxb)	Indian Botanic Garden, Howrah
2	*B. corymbosa* Roxb ex DC	-do-
3	*B. diphylla* Buch Ham	-do-
4	*B. galpinii* N E Brown	-do-
5	*B. hookeri* F Muell	-do-
6	*B. petersiana* Bolle	-do-
7	*B. ferruginae* Roxb	-do-
8	*B. retusa* Buch Ham ex Roxb (= *B. roxburghiana* Voigt)	-do-
9	*B. rufescens* Lam	-do-
10	*B. tomentosa* L	Chikkadpally, Hyderabad
11	*B. vahlii* Wight & Arn	Indian Botanic Garden, Howrah
12	*B. variegata* L var *alboflava* De Wit	Public Garden, Hyderabad
13	*B. variegata* L var *variegata* De Wit	Osmania Univ. Campus, Hyderabad

graphed under identical laboratory conditions. The unidentified designated by their hRf (100 × Rf) values.

3. Observations

3.1 *Secondary metabolites*

The distribution of various secondary metabolites presented in table absence of alkaloids, aucubin compounds, cyanogenic glycosides, and tannins and uniform occurrence of similar broad flavonoid syringyl radicals and triterpenoids. However, there is restricted distr other chemical compounds in the taxa studied. Thus the anthraquino *B. galpinii*, *B. hookeri* and *B. scandens* var *horsfieldii*; aurones

Table 2. Distribution of secondary metabolites.

hemical	Name of the taxon*												
onstituent	1	2	3	4	5	6	7	8	9	10	11	12	1
lkaloids	–	–	–	–	–	–	–	–	–	–	–	–	
nthraquinones	+	–	–	+	+	–	–	–	–	–	–	–	
ucubin compounds	–	–	–	–	–	–	–	–	–	–	–	–	
urones	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	N
atechol-tannins	+	–	+	+	+	–	–	–	–	+	+	–	
yanogenic glycosides	–	–	–	–	–	–	–	–	–	–	–	–	
llagic acid	+	+	+	+	+	–	–	+	–	+	+	+	
lavonoids	+	+	+	+	+	+	+	+	+	+	+	+	
ndoles	–	–	–	–	–	–	–	–	–	–	–	+	
uglone	–	–	–	–	–	+	+	–	+	–	–	–	
eucoanthocyanins	+	+	+	+	+	+	+	+	+	–	+	+	
ignans	–	–	–	–	–	–	–	–	–	–	–	–	
lethylene dioxy compounds	–	–	–	+	–	+	+	–	–	+	–	–	
henols	+	+	+	+	+	+	+	+	+	+	+	+	
aponins	–	+	–	–	–	–	–	–	–	–	–	–	
teroids	+	+	–	+	+	–	+	+	+	–	+	+	
yringin	–	–	–	–	–	–	–	–	–	–	–	–	
yringyl radicals	+	+	+	+	+	+	+	+	+	+	+	+	
annins	–	–	–	–	–	–	–	–	–	–	–	–	
riterpenoids	+	+	+	+	+	+	+	+	+	+	+	+	
ctivity of Polyphenolase	+	+	+	–	–	+	+	+	+	+	–	+	

same as in table 1; + = positive; – = negative; NA = not applicable

2 *Free aminoacids*

he distribution pattern of the free amino acids (protein and nonprotein) is presente table 3. Out of a total of 23 amino acids in the free pool 18 could be identified rotein amino acids and the remaining 5, presumably the nonprotein ones, a esignated by their hRf values. Of the several identified protein amino acids in the fr ool, threonine seems to be more common. Its presence is noticed in all the taxa exce . *vahlii*. Tyrosin occupies the second position in distribution. It is present in all t xa except *B. corymbosa*, *B. diphylla*, *B. ferruginae* and *B. galpinii*. Methionine a utamine are spotted in 8 out of the 13 taxa studied. The rest of the amino acids ha estricted distribution in the free pool. Thus, alanine could be seen in *B. diphyl*

Table 3. Distribution of amino acids.

Aminoacid	Name of the taxon*									
	1	2	3	4	5	6	7	8	9	1
Alanine	+		+		+	+	+	+		
γ-amino butyric acid		+	+	+		+	+	+	+	+
Aspartic acid										
Glutamic acid							+	+	+	
Glutamine	+	+	+		+	+	+			
Glycine	+								+	
Histidine				+						
Leucine				+		+			+	+
Isoleucine							+			
Norleucine				+						
Lysine			+	+				+		
Methionine	+	+	+	+	+				+	
Phenylalanine			+					+		
Serine		+								
Threonine	+	+	+	+	+	+	+	+	+	+
Tryptophan				+	+					
Tyrosine	+				+	+		+	+	
Valine	+		+		+					
hRf 20				+	+		+	+	+	
hRf 26						+				
hRf 28									+	
hRf 30	+	+	+					+		
hRf 50					+		+			

* same as in table 1.

members except *B. corymbosa*, *B. diphylla*, *B. petersiana*, *B. scandens* *B. tomentosa* and *B. variegata* var *variegata*. The other unidentified ar as hRf 26 (in *B. petersiana* and *B. vahlii*) hRf 28 (in *B. rufescens* and bo of *B. variegata*) hRf 30 (in *B. corymbosa*, *B. diphylla*, *B. retusa*, *B* *horsfieldii* and *B. tomentosa*) and hRf 50 (in *B. ferruginae* and *B. hooke* distribution.

4. Discussion

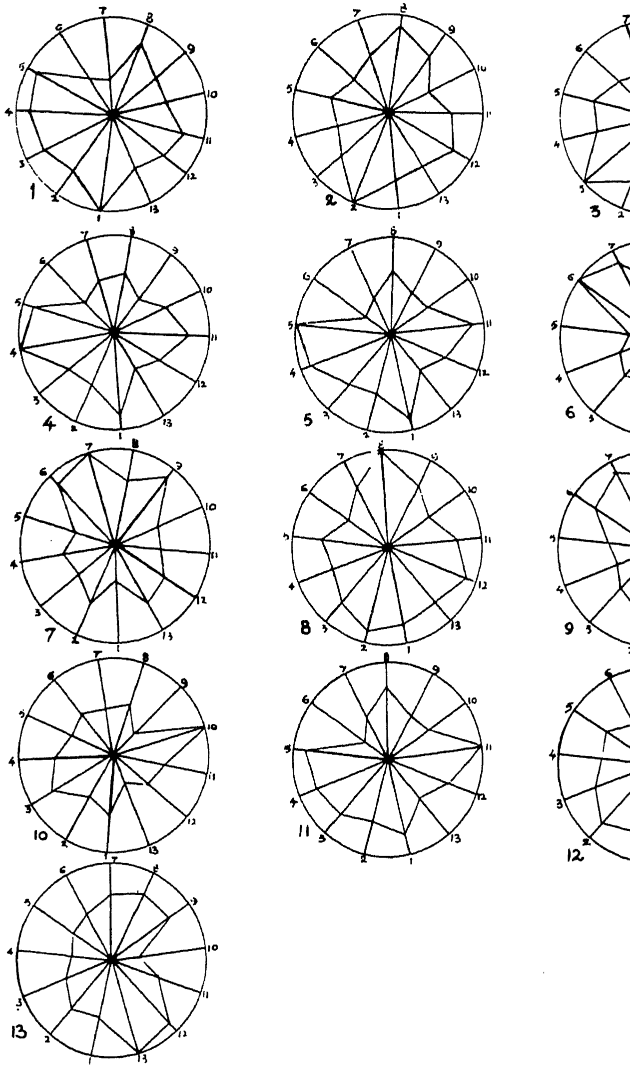
dicals and triterpenoids and the absence of a few other chemical constituents as statec ie preceding paragraphs. The uniform presence or absence of a particular compour course, has little taxonomic significance. Further the similarities in the negat haracters are to be ignored (Runemark 1968). Thus the paired affinity indices lculated on the basis of distribution of the remaining chemical compounds, clos llowing Ellison *et al* (1962) and employing the positive matches and the differen able 4). They are further depicted in the polygonal graphs (Hutchinson 1936) gures 1–13 to indicate the degree of affinity at a glance. The summation of pair finity indices known as group affinity index (GAI) (Ellison *et al* 1962) expresses t finity of one taxon with all others. Thus GAI should be 1300 in the present contex ere is 100 % affinity and 100 if it is least. The GAI (table 4) which range from 671–9 so speak of close chemical ties among the taxa studied.

Bate-Smith (1962) distinguished the species and the families of dicotyledons ir ur classes on the basis of presence or absence of leucoanthocyanins (a, a_0) a ihydroxy constituent, the ellagic acid (b, b_0) and found the trend of evolution to be e direction of $ab \rightarrow a_0b_0$ with transitional forms a_0b and ab_0. Viewed from this an majority of the species of *Bauhinia* (present study) are to be regarded as primit b class), *B. yunnanensis* (Bate-Smith 1962) as highly advanced (a_0b_0 class) a *tomentosa* (a_0b class), *B. ferruginae*, *B. petersiana*, *B. rufescens* and *B. variegata* v *iriegata* (ab_0 class) (present study) forming the evolutionary bridge.

The protein amino acids are of ubiquitous occurrence, but they exhibit considera iantitative variation depending upon the metabolic threshold of the tissue a ivironmental conditions. Hence their distribution pattern in the free pool, has lit xonomic significance. The nonprotein amino acids, on the other hand, are n iiversally present and their incidence has relevance in taxonomic considerations at vels of hierarchy up to the family (Gershenzon and Mabry 1983). The species *auhinia* have been assigned to four different genera *viz Lasiobema* (Korth) M *hanera* Lour, *Piliostigma* Hochst and *Bauhinia* L (*s. s.*). The commonness to a la

Table 4. Paired and group affinity values.

ime of the xon*	Name of the taxon*													Group affinity
	1	2	3	4	5	6	7	8	9	10	11	12	13	
	100	72	80	83	90	40	36	80	60	60	80	72	60	913



Figures 1–13. Polygonal representation of the paired affinity value
mentioned in table 4) to all others. Affinity values are expressed along th
beginning at the centre.

The species now studied could be identified on the basis of some of the chemi
aracters.

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collect the live material of some of the taxa from the Indian Botanic Gard
owrah and for their identification and to Prof. G Weimark for helpful suggestio
N and MR thank Prof. M V Pattabhiraman for his interest and UGC unit of Osma
niversity for financial assistance.

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tomatal studies in Amaryllidaceae with special reference o stomatal abnormalities

D K AWASTHI, V KUMAR* and R RAWAT

Department of Botany, M M Post Graduate College, Modinagar 201 204, India
*Department of Botany, Meerut University, Meerut 250 005, India

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Abstract. Foliar epidermis of nineteen species of Amaryllidaceae, has been stud Epidermal cells on both the surfaces are polygonal with oblique or straight anticlinal wall all species except in *Haemanthus* and *Eucharis* where these have sinuous or undulate wa Subsidiaries are indistinct and are of *C*-type. With the exception of *Eucharis* (hypostoma leaves are amphistomatic in all the species. The stomata are anomocytic. Various types stomatal abnormalities *viz* contiguous stomata, interstomatal cytoplasmic connections etc., observed in the different species.

Keywords. Amaryllidaceae; foliar epidermis; monocotyledons; stomata.

. Introduction

he variation and organization of stomatal complex of foliar epidermis of monoco dons has been given considerable phylogenetic importance (Stebbins and Khu 961; Tomlinson 1974). Stebbins and Khush (1961) reported anomocytic stoma hereas Shah and Gopal (1970), reported two types—the anomocytic and t aracytic—in a few members of the Amaryllidaceae. This has necessitated an extensi vestigation of the stomatal complex so as to assess the types of stomata occurring ie Amaryllidaceae.

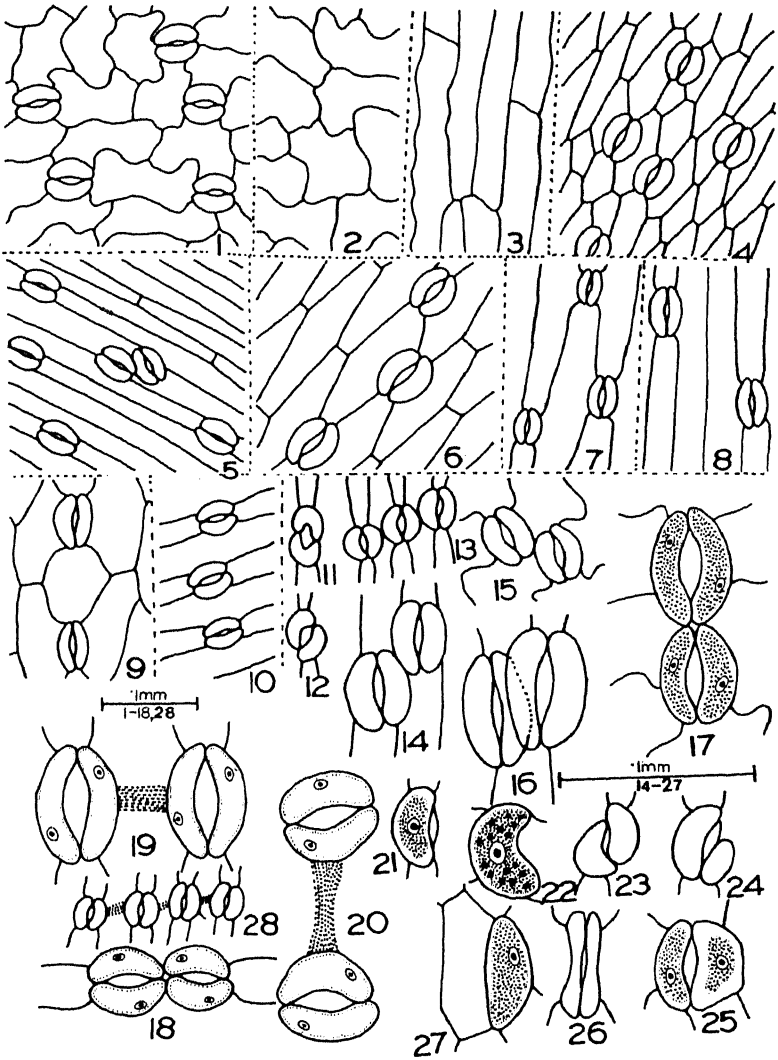
. Materials and methods

he epidermal peels were obtained from young and mature leaves of the followi pecies: *Agapanthus umbellatus* L. Her, *Allium cepa* L, *A. sativa* L, *A. tuberosum* Ro

Haemanthus multiflorus and *Eucharis grandiflora* where they are sinuc
having distinct costal areas. The epidermal cells in the costal area ar
undulate walls (figure 3). The frequency of the epidermal cells per mı
23·20 ± 3·15–301·60 ± 18 and 26·4 ± 4·29–359·20 ± 8·59 on the upper
faces respectively. The length of the epidermal cells is also variable.
164·01 ± 26·49 μm (*Agapanthus umbellatus*) to 304·59 ± 45·10 μm (*Alliu*
upper surface and 121·44 ± 20·50 μm (*Amaryllis hybrida*) to 301·95 ± 5
cepa) on the lower surface. On both the surfaces, epidermal cells are
uniformly thick cuticle.

In all the species the stomata are anomocytic (Metcalfe and Chalk 19
stomata are uniformly distributed in the inter-costal areas and are orie
the long axis of the epidermal cells. Smith (1935) also suggested a
orientation of stomata in general for monocotyledons. In *A. sativa* (*L*
(*L*), *Amaryllus belladonna* (*L*), *N. curvifolia* (*U*, *L*), *Cyrtanthus ma*
Haemanthus multiflorus (*L*) a few stomata are either oblique or at rig
long axis of the epidermal cells (figures 11, 12, 23). The stomata are sepa
many intervening cells and are surrounded by 3–5 subsidiary cells whic
from the epidermal cells. The leaves are hypostomatic in *Eucharis gran*
the rest of the species they are amphistomatic. The stomatal fr
appreciably in different species but no significant variation is observed i
of the same leaf. The stomatal frequency varies from 8·80 ± 3·15 (
multiflorus) to 83·20 ± 41·43 (in *Allium sativa*) per mm^2 area on the up
9·60 ± 2·06 (in *Zephyranthes candida*) to 103·20 ± 13·70 (in *Allium sativ*
on the lower surface. The stomatal index varies from 7·82 (in *Amaryllis h*
(in *Allium tuberosum*) on the upper and 9·44 (in *Zephyranthes candi*
Allium sativa) on the lower surface. The size of the stomata ranges betw
× 28·71 ± 1·50 μm and 57·09 ± 5·40 × 38·61 ± 2·22 μm in different spe

Two types of contiguous stomata were observed (a) laterally cont
seen in *Agapanthus umbellatus* (*L*), *Allium cepa* (*L*), *A. tuberosum*
belladonna (*U*, *L*), *A. vittata* (*U*, *L*), *Nerine curvifolia* (*L*), *Crinum zey*
C. asiaticum (*L*), *Cyrtanthus mackenii* (*L*), *Cooperia pedunculata* (*U*,
verecundum (*L*), *Narcissus tazetta* (*U*) etc., (figures 13–16) where
contiguous stomata were found only in *Allium cepa* (*L*), *Allium*
Amaryllis belladonna (*L*), *Haemanthus multiflorus* (*L*) and *Narcissus taz*
17, 18). The laterally contiguous stomata are more frequent than the pol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1-18,28
·1mm
14-27

(*L*) (figure 25). Stomata with a single guard cell which occur in *A*
(*U*), *Nerine curvifolia* (*U*, *L*), *Crinum album* (*L*), *Cyrtanthus macken*
candida (*U*), *Haemanthus multiflorus* (*L*), *Pancratium verecundum*
tazetta (*U*) (figures 22, 27) are without a pore but others observed
Amaryllis belladonna (*L*), and *Cooperia pedunculata* (*L*) have
Stomata with degenerated guard cells were observed in *Allium cepa*
and *Pancratium verecundum* (*L*) (figure 26).

4. Discussion

In a majority of the species investigated the epidermal cells are poly
with oblique or straight anticlinal walls with the exception of
Haemantheae) and *Eucharis* (tribe-Eucharideae). In these instan
have sinuous or undulated walls.

In this paper the term subsidiary cell has been used for a cell surro
irrespective of the fact whether it is distinct or indistinct. There
regarding the presence or the absence of subsidiaries in monocot
1974). According to Stebbins and Khush (1961), Paliwal (1969) a
subsidiary cells are absent in Amaryllidaceae. Usually one indisti
common for 2–4 stomata (figures 6, 7, 9). Such type of subsidiaries a
Ramayya and Rajgopal (1980). According to them such subsidiar
both the surfaces in those taxa in which the costal cells are ind
observations fully substantiate their views.

In the Amaryllidaceae, Shah and Gopal (1970) recorded both the
paracytic types. But the two genera, *Agave* and *Curculigo*, where pa
observed are treated as a separate family—Agavaceae (Hutchinson
the basis of the present observation and those of earlier workers (
1961; Shah and Gopal 1970), it is concluded that the stomata are in
in Amaryllidaceae.

Stebbins and Khush (1961) reported the presence of anomo
majority of Liliales and allied orders, the Iridales, Amaryllidales et
reported anomocytic type of stomata in Iridaceae. According to
there is a close affinity between Liliales, Iridales and Amaryll
observations support the ideas of Hutchinson. Although Shah
reported some sort of connections between guard cells of two stoma

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x reversal and fruit formation on male plants of *ırica Papaya* L by ethrel and chlorflurenol

ARAVIND KUMAR* and V S JAISWAL

Department of Botany, Banaras Hindu University, Varanasi 221 005, India
* Present Address: Department of Botany, Government Post-Graduate College, Gopeshw
(Chamoli) 246 401, India

MS received 27 April 1984; revised 20 August 1984

Abstract. Male plants of *Carica papaya* L were induced to bear female flowers and yield good fruit crop by the application of ethrel and chlorflurenol at various concentration During conversion of male flowers into female flowers, some intersexual flowers wi transition were observed. The female flowers produced by ethrel were similar to the contr female flowers whereas those produced by chlorflurenol were abnormal.

Keywords. *Carica papaya*; chlorflurenol; ethrel; sex-expression.

Introduction

paya (*Carica papaya* L.) is a tropical plant, cultivated for its large, delicious fruit e sex of an individual papaya plant can be identified only after the appearance of flowers. If the plant bears male flowers, the grower has no other option except to cu lown. There are reports regarding the effects of a few environmental factors an tain chemicals on its flowering behaviour (Storey 1953; Lang 1961; Singh *et al* 1963 ose and Sen (1975) and Jindal and Singh (1976) found greater percentage of femal nts in the population by exogenous application of a few growth regulators a dling/sapling stage. However, induction of female flowers on genetically male plant papaya has not been achieved so far.

Ethrel (2-Chloroethylphosphonic acid) and chlorflurenol (2-chloro-9-hydroxy rene (9) carboxylic acid) have been shown to produce female flowers in male plants c *nnabis sativa* (Mohan Ram and Jaiswal 1970, 1971) and *Morus nigra* (Jaiswal an mar 1980, 1981). These growth regulators were therefore applied to male plants c rica papaya to study their role in sex reversal and fruit formation.

grown plants bearing a few male flowers whereas similar control plan
0.1 % of the surfactant. Second application of each concentration of bo
was made on the respective plants after a fortnight.

3. Results

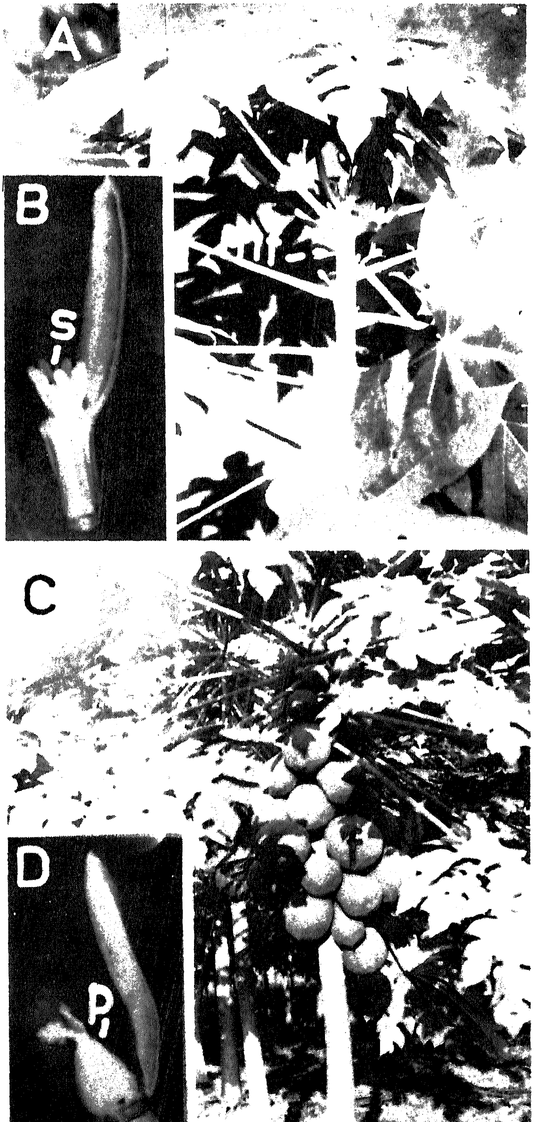
3.1 *Effects of ethrel on sex expression*

Control male and female plants produced male and female flowe
However, ethrel treatment at all concentrations produced female an
flowers with or without male flowers. As a result of ethrel treatment
30 % of the plants produced only female flowers, 20 % produced female
flowers and 25 % produced flowers of all the three sexes (*i.e.* ma
intersexual). On 5 % plants, no female or intersexual flowers were obse
the remaining 20 % plants, only male and intersexual flowers deve
Likewise at 480 mg l^{-1} of the chemical, the percentage of plants produ
flowers, female and intersexual flowers and flowers of all the three se
less same as that observed at 240 mg l^{-1}. Nevertheless, at 960 mg l^{-1}, 3
produced only female flowers, another 35 % produced both female
flowers and the remaining plants produced flowers of all the three s

During feminization, several transitional stages of flower sex
Feminization started with increase in the size of the young flower bu
of green colouration with stout base of the flower buds and asymme
stamens (figure 2 A, B, C). Filaments of most of the stamens in each flo
flat structures bearing pollenless anthers (figure 2C). In fully transfor
trace of anthers was noticed. Along with the reduction in the pollen-be
the anthers, simultaneous development of the pistil appeared. In the ini
filamentous structures with flat apices (generally 2–5) emerged from t
receptacle and grew longer than the stamens protruding out of the flo
In advanced stages, the base of the pistil became bulb-like with a flat a
Highly feminized intersexual flowers had fully-developed pistils with p

Table 1. Effect of ethrel and chlorflurenol on the male plants of *C. p*

	Percentage of plants producing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B
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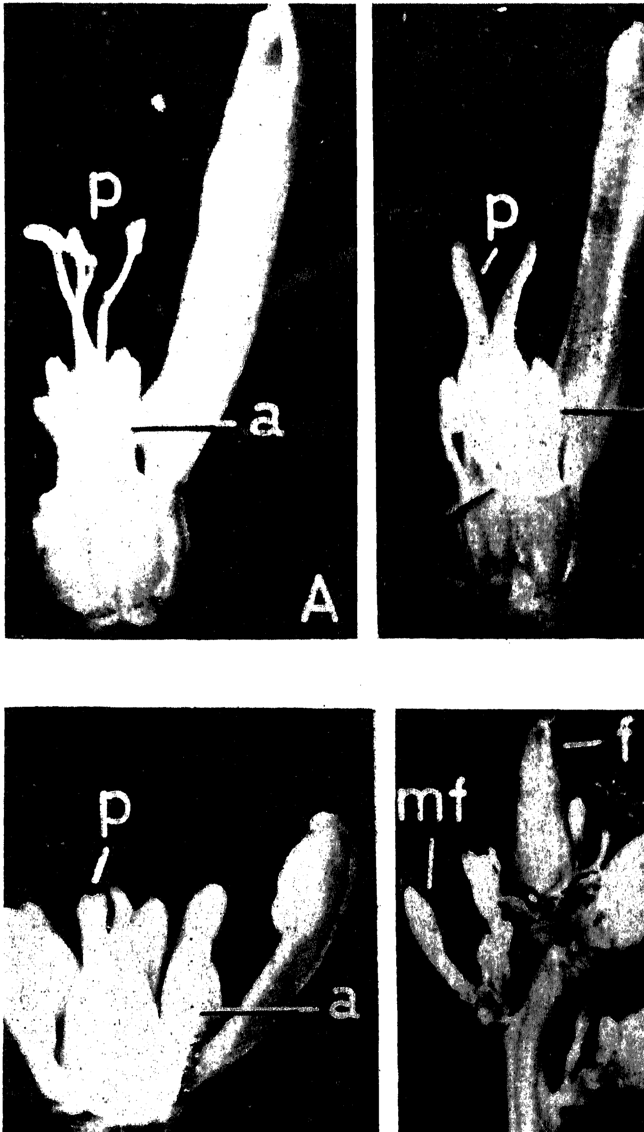
D
P


p
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A
p
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of chlorflurenol on sex expression

chlorflurenol treatment, 5 % of the plants at 40 mg l^{-1} and 10 % of the plants $^{-1}$ were entirely burnt out. Among the remaining plants, 20 % at 20 mg l^{-1}, mg l^{-1} and 55 % at 80 mg l^{-1} of the chemical produced only female flowers he number of plants bearing male and intersexual flowers was 15 % at 20 and and 5 % at 80 mg l^{-1} whereas those bearing intersexual and female flowers t 20 mg l^{-1} and 15 % at 40 and 80 mg l^{-1} (table 1). The nature of intersexual s similar to that observed in response to ethrel-treatment.

Control female plants usually bear a single fruit in each lea However, in the chlorflurenol-treated plants, they were formed in Greater number of fruits were produced on almost all plants which v sugar content and colour of the flesh as compared to control. Howe quite abnormal in shape (figure 3). Some fruits in cross-section tetralocular cavity (figure 3B) whereas some fruits were solid inside with normal cavity and aborted ovules were also observed (figure 3 seeds were enclosed in a homogeneous glossy, gelatinous coating induced fruits, some seeds lacked such covering while others had a hard coating. A few seeds were found attached to 5–55 mm long Vivipary was observed in many fruits.

4. Discussion

The present observations clearly demonstrate the production of bear fruits in male plants of *C. papaya* by ethrel and chlorflurenol Ghose and Sen (1975) and Jindal and Singh (1976) had reported tha application of seedlings of *C. papaya* increased the percentage of f population and not induction of female flowers on genetically ma

Ethrel releases ethylene into the plant tissue which increases it (Cooke and Randall 1968). A higher level of ethylene favours fem (Abeles 1973; Lieberman 1979). It has been observed that once e threshold level of ethylene inside the plant tissue of *C. papaya*, t producing female flowers gradually increased and the treated plan male flowers.

Chlorflurenol, a synthetic chemical modifies the sex expres interacting with endogenous growth hormones (Parups 1980). Auxi feminity-inducing hormone (Frankel and Galun 1977). Since chlorfl the translocation of auxin (Gagianas and Burg 1977), the latter ac place of its origin/synthesis in plants treated with chlorflurenol. T conjectured that in *C. papaya* auxin synthesized near the growin primordia develop) does not move from the place of its orig chlorflurenol treatment and its accumulation in greater amount developing primordium controls the fate of the same.

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armacognostic studies on the flower of *Calophyllum inophyllun* nn

SHANTA MEHROTRA, USHA SHOME and H P SHARMA

Pharmacognosy Laboratory, National Botanical Research Institute,
Lucknow 226 001, India

MS received 24 May 1983; revised 13 June 1984

Abstract. *Calophyllum inophyllum* Linn is one of the important Ayurvedic drug plants c which practically all parts are used medicinally. Among these flowers and stamens are ofte used as a substitute for 'Nagkesara'. This paper deals with a detailed pharmacognosy of th flower of *C. inophyllum*. Physico-chemical constants, preliminary phytochemical studie fluorescence analysis, behaviour of powdered drug with different chemical reagents and thi layer chromatography were also carried out.

Keywords. *Calophyllum inophyllum*; flower; pharmacognosy; Cluseaceae.

Introduction

ophyllum inophyllum Linn (family-Cluseaceae) which is variously known as Und ndi); Sultan champha (Bengali); Nagchampa, Nagkesara (Sanskrit) and Alexende ırel (English) is an important Ayurvedic drug plant and is used as a substitute fo gkesara' (Chunekar 1960; Shaligram 1953; Singh and Chunekar 1972). Differen ts of this plant are variously valued for their digestive, antipyretic, diaphoretic an idysentric properties. The decoction of the flowers taken internally, is said to cur hilitic eczema and insanity (Rao 1914).

Calophyllum inophyllum is an ornamental tree with a thick trunk and smooth gre k, large smooth shining leaves with white sweet scented flowers, occurring along th stal regions of the southern India, Andaman Islands, Burma and Ceylon.

good amount of work on the chemistry of the seed oil, bark, heart wood and leave *C. inophyllum* has been carried out by various workers. Subramanium and Nai 65, 1971) isolated myricetin-7-glucoside from the androecium of the flower, th ıcture of which was elucidated by Kasim *et al* (1974).

fair amount of work has also been carried out on several botanical aspects of th

stained with safranin and fast green were used for histological study petals and sepals were treated with a dilute solution of nitric acid fo in chloral hydrate and lactic acid. Physicochemical studies wer shade-dried material.

3. Gross morphology of the flower

The flowers are fragrant; white; pedicellate; pedicel 1–2 cm, slende solitary or fasicled racemes (figure 1).

Sepals 4, reflexed, two outer ones concave (figure 6), inner petaloi imbricate and depressed-globose (figure 24). Stamens indefinite, base, filaments small, anthers oblong; ovary superior, unicarpellary short, slender; stigma capitate (figures 43–45), ovule single, erect.

A young fruit is green to yellow but the mature fruit is brown in c shortly acuminate and seed ovoid or globose.

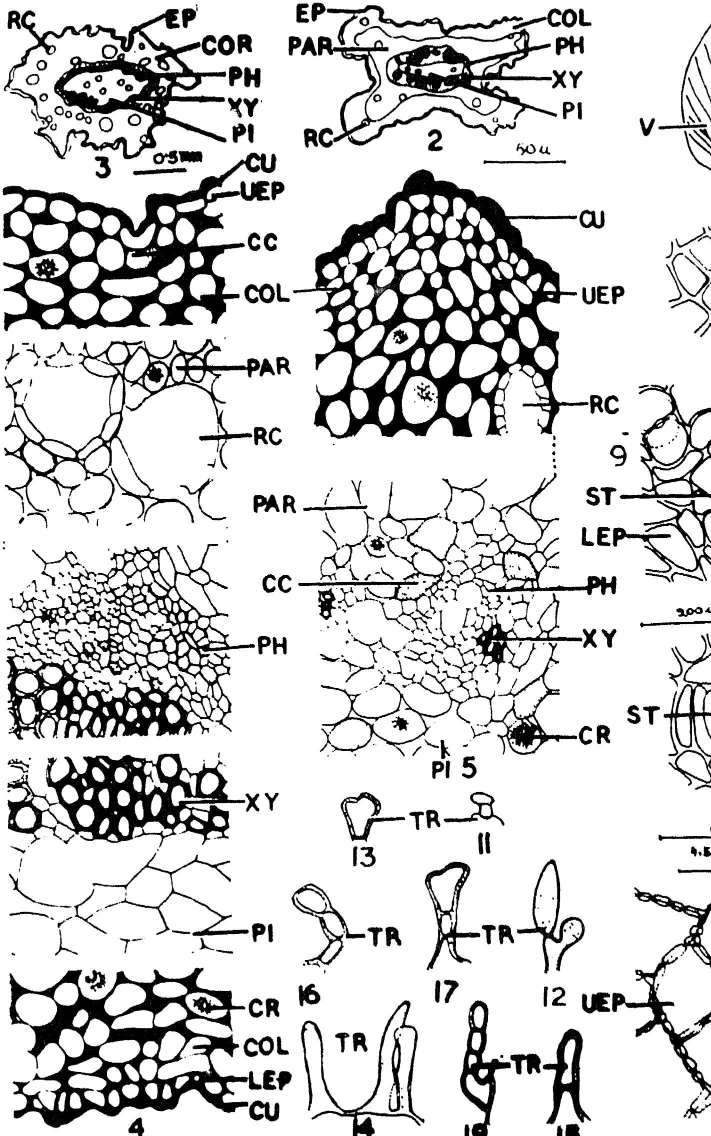
4. Microscopic characters

4.1 *Pedicel*

A TS of the pedicel is squarish or round to oval in shape (figures 2 single-layered epidermis with a fairly thick crenate cuticle. The c cortex are collenchymatous, their number being greatly variable Schizogenous resin canals and prismatic crystals of calcium oxalate the cortex. The vascular cylinder is an amphiphloic siphonostele wi in groups (figures 2 and 3). The xylem is endarch and comprises of fibres and xylem parenchyma. The phloem is well-developed and tubes, companion cells and phloem parenchyma. Most of these c yellowish brown pigments (figures 4 and 5). The pith is parench schizogenous resin canals (figures 2–5).

4.2 *Calyx*

The epidermal cells of the adaxial surface of the outer sepals are walled (figure 7) and devoid of stomata. However, those of the inne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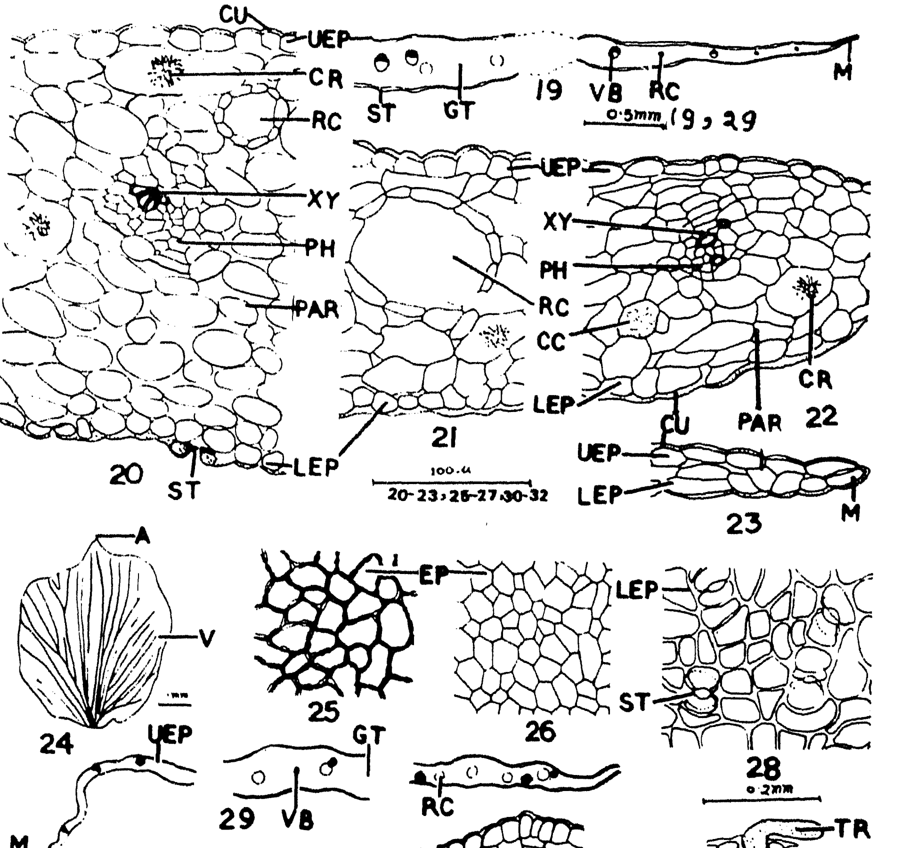
pals. However, adaxial epidermal cells of the inner petals are uniform, hexagona n-walled and devoid of stomata (figure 26). There are no glandular hairs on th pals but non-glandular ones, which are uni-celled, with slightly bulging bases an unded apices are present along the margins (figure 27).

Epidermal cells on the abaxial side are variable and have sunken, paracytic stoma e the sepals (figure 28). However, the frequency, and size of stomata are muc aller here and the ledges are thin-walled.



The TS of the petal is irregular in shape (figure 29). The parenchymatous and is 2–3 layers thick at margins (figure 30) and layers in the middle (figure 31). The vascular bundles are prese distribution of the xylem and phloem is irregular (figures 29, 31 anc schizogenous resin canals are distributed throughout the ground t

4.4 *Androecium*

The numerous stamens have short thick filaments and elongated an 33 and 34) as in *Mesua ferrea* (Shome *et al* 1982). The stamens are fus separate upwards (figures 35 and 36). Their staminal bundles (figure 37).

A semi-diagrammatic representation of the TS of anther lobes is sl The surface cells of the anther lobes are thick-walled and show close (figure 39).

4.5 *Pollen grain*

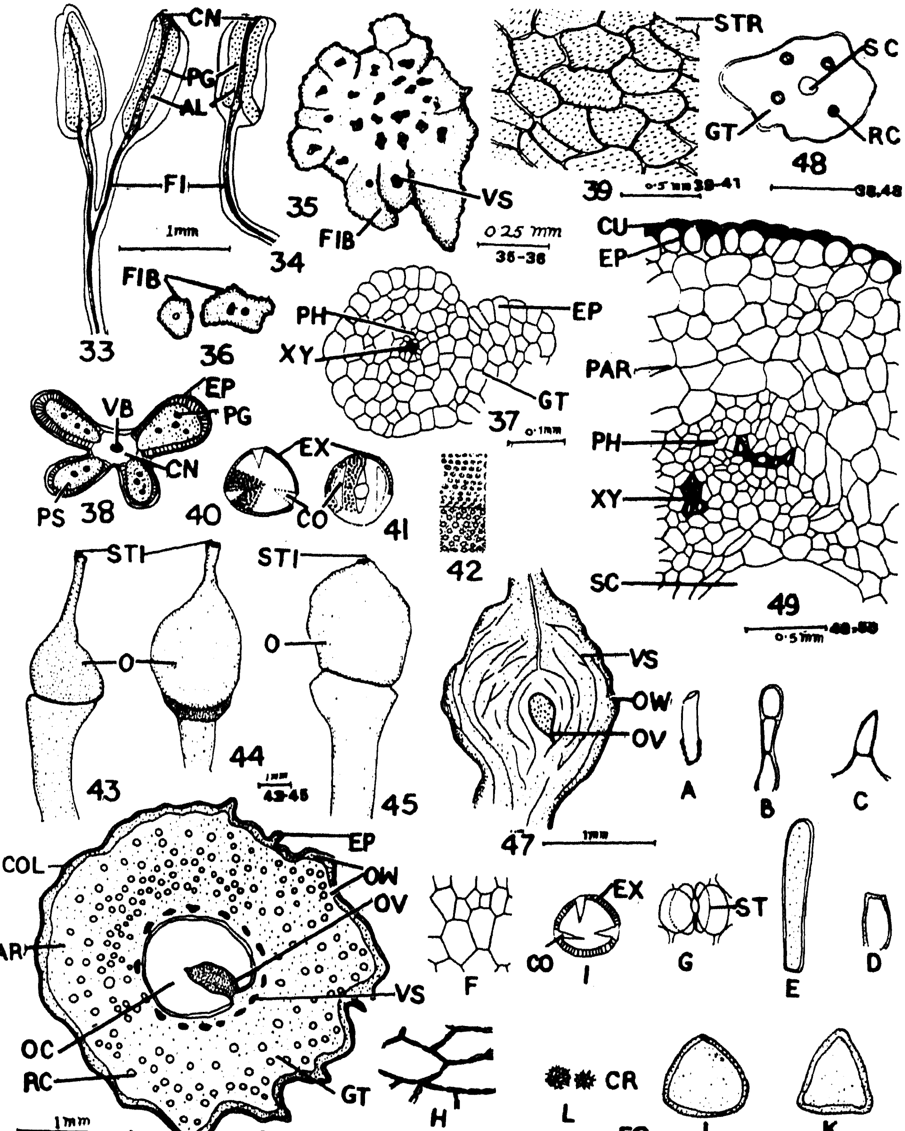
Pollen grains are 2–(3–) colporate (average size 34·62 × 3 30·47–38·78 × 27·77–36·01); shape subolate-prolate, spheroidal reticulate; thickness 1–2 μm, muriduplibaculate, colpus measuri (figures 40–42).

4.6 *Gynoecium*

The ovary is superior and monocarpellary (figure 46). It has a single- and many-layered ground tissue. The latter is differentiated into an collenchymatous region filled with dark brown pigments and an inn parenchymatous region. Schizogenous resin canals are present all c submarginal and anatropous (figures 46 and 47).

A TS passing through the style shows a stylar canal in the centre anc all round (figure 48). The epidermis is single-layered and has a t ground tissue is parenchymatous and the vascular bundle is amphic

5. Powder study



CN
PG
AL
FI
1mm
33
34
35
VS
FIB
0.25 mm
35-36
FIB
36
STR
39
0.5 mm 39-41
SC
GT
RC
48
38,48
CU
EP
PAR
PH
XY
SC
49
0.5 mm
PH
XY
EP
GT
37
0.1mm
VB
EP
PG
CN
PS
38
EX
CO
40
41
42
STI
O
43
44
1mm
43-45
STI
O
45
VS
OW
OV
47
1mm
A
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C
EP
COL
OW
OV
AR
VS
OC
RC
GT
1mm
F
EX
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I
ST
G
E
D
H
CR
L
J
K

Table 1. Behaviour of powder on treatment with different chemical reagents.

Powder +	Observations
Powder as such	Light brown
Picric acid	Brown
Acetic acid	Dark brown
Conc. HCl	Blackish brown
Conc. H_2SO_4	Light brown
Iodine (5 %)	Dark brown
Ferric chloride (5 %)	Greenish black
Sudan III	Red
Conc. HNO_3 + ammonia	Orange with black tinge

Table 2. Fluorescence analysis of the powdered drug.

Powder +	Colour in ordinary diffused light	Fluorescence under UV-light
Drug as such	Greyish brown	Brown
Nitrocellulose in amylacetate	Dark brown	Dark brown
1N HCl	Brown	Greenish brown
1N HCl + nitrocellulose in amylacetate	Brown	Brown
1N NaOH (aqueous)	Dark brown	Blackish brown
1N NaOH (aqueous) + nitrocellulose in amylacetate	Blackish brown	Bright greenish brown
1N NaOH in methanol	Blackish brown	Greenish brown
1N NaOH in methanol + nitrocellulose in amylacetate	Blackish brown	Greenish brown
50 % Nitric acid	Dark brown	Dark purple

Table 3. Determination of physico-chemical constants on air dry weight basis.

Constants	Percentage
Ash	
Total	2·9389
Acid insoluble	0·169
Tannins	10·6
Sugars (mg/g dry wt)	
Water soluble	53
Acid insoluble	67
Extractives	
Alcohol soluble	17
Water soluble	18

Table 4. Phytochemical tests in *C. inophyllum* Linn.

	Extractives				
rameters	Hexane	Benzene	Chloroform	Ethanol	Aqueous
tal percentage weight	3·883 %	1·585 %	0·747 %	10·974 %	11·759 %
ysical pearance and nsistency	Greenish brown, waxy, very pleasant smell	Greenish brown, brittle, pleasant smell	Greenish brown, waxy, pleasant smell	Dark brown, tachy, sugary smell resembling Drakshasava	Dark brow brittle
kaloids	–	–	+	+	–
avonoids	–	–	–	+ + +	+ +
ducing sugars	–	–	–	+ + +	+ + +
sins	+	+ +	±	–	–
ponins	–	–	–	–	+
eroids	+ + +	+	–	–	–
iterpenoids	–	–	–	–	–
nnins	–	–	+	+ + +	+ +

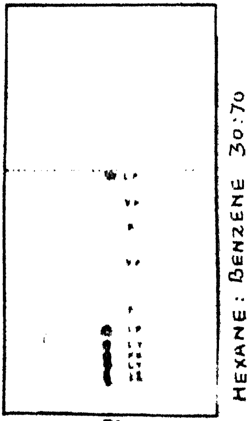
tal percentage of Extractive = 28·948 %

6.1 *Hexane extractive*

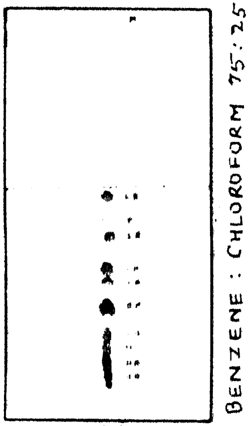
As many as 11 spots were discernible in unidirectional thin layer using hexane : benzene (30 : 70) as the solvent system. Out of th prominent and 4 faint to very faint (figure 51).

6.2 *Benzene extractive*

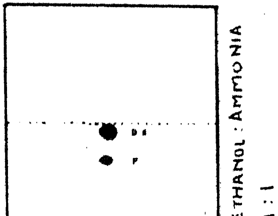
Using benzene : chloroform (75 : 25) as the solvent system, benzene e spots. Excepting one spot near the solvent front, the others were quit of these one spot nearer the starting line is characteristically arrow- purple in colour (figure 52).

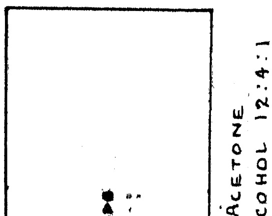


51
HEXANE EXTRACTIVE



52
BENZENE EXTRACTIVE





3 *Chloroform extractive*

he chloroform extractive resolved into 5 clear spots and a trail near the start w lloroform : methanol : ammonia (85 : 14 : 1) as the solvent system (figure 53).

4 *Alcohol extractive*

he alcoholic fraction gave 12 clear spots in the solvent system chloroform : aceto propyl alcohol (12 : 4 : 1) (figure 54).

The hRf values of different extractives in various solvent systems are presen table 5.

Discussion

inophyllum L belonging to the 'Nagkesara' (*Mesua ferrea*) family may be call unnaga' and its flower buds are at present being used in place of real 'Nagkesa ingh and Chunekar 1972). The genuine 'Nagkesara' is unanimously accepted to *'esua ferrea* Linn (Dymock *et al* 1890; Satkopan and Thomas 1967; Vaidya 19 'aring 1868). The pharmacognosy of the genuine drug was carried out earlier (Sho *al* 1982). The present study is an attempt to describe its substitute—*C. inophylli* nn and to differentiate it from the genuine one. It has brought out significa fferences particularly in certain characters of pedicel, such as (i) absence of semilur ickenings in the cortex (ii) presence of starch grains in the innermost one or t yers again of the cortex (iii) presence of pericyclic fibres and (iv) abundant rose ystals of Ca-oxalate in the pith. The other characters which can be profitably used stinguish the two are presence of glandular hairs on non-essential parts of the flov ıd unicarpellary ovary.

C. inophyllum also differs markedly from *Mesua ferrea* L in having a low percenta 'ash 2·938, high percentage of tannin 10·6 (ash value and tannin percentage of *Mes rrea* being 9·297 and 4·4 respectively) and presence of steroids and flavonoids whi e totally absent in *Mesua ferrea* Linn. The aforesaid characters may thus be used fferentiate the genuine 'Nagkesara' from its substitute.

Table 5. hRf values in C. inophyllum Lin

The authors are thankful to the Scientist-in-charge for facilities. Th
Messrs M K Tandon and A Jha for technical assistance.

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Abbreviations: *A*, apex; *Al*, anther lobe; *C*, cell contents; *CN*, connective; *CO*, colpus *COR*, cortex; *CR*, crystal; *CU*, cuticle; *EP*, epidermis; *EX*, exine; *FI*, filament; *FIB* ground tissue; *LEP*, lower epidermis; *M*, margin; *O*, ovary; *OV*, ovule; *OW*, ovary wal *PG*, pollen grain; *PH*, phloem; *PS*, pollen sac; *RC*, resin canal; *SC*, stylar canal, *ST*, s *STR*, striations; *TR*, trichome; *UEP*, upper epidermis; *V*, vein; *VB*, vascular bundle; *XY*, xylem

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licropropagation of *Salix babylonica* through *vitro* shoot proliferation

K K DHIR, RAJIV ANGRISH and MONIKA BAJAJ
Department of Botany, Panjab University, Chandigarh 160 014, India

MS received 22 July 1983; revised 15 September 1984

Abstract. Shoots originating from nodal stem explants of *Salix babylonica* showed maxim proliferation through axillary branching on a modified Murashige-Skoog medium su plemented with 1 mg/l of benzylaminopurine. Incorporation of naphthaleneacetic acid gibberellic acid did not enhance shoot proliferation in the presence of benzylaminopuri Shoots were grown to a suitable length on a medium with low concentration of b zylaminopurine. Subsequently they were excised and allowed to root on a basal medium, w or without a short pre-treatment with naphthaleneacetic acid. Rooted plantlets w transferred to pots and maintained under high humidity conditions to prevent th desiccation. The high humidity conditions were gradually withdrawn and the plantl established as independently growing saplings.

Keywords. *Salix babylonica*; micropropagation; *in vitro* shoot proliferation.

Introduction

the past few years micropropagation of woody plants through *in vitro* sho oliferation has gained increasing attention. Generally shoot apices are cultured or tokinin supplemented medium. This results in reduced apical dominance along t oot axis and encourages the growth of axillary buds into lateral branches. Tl ethod is direct as it avoids the callus/suspension stages that often are morphogene lly recalcitrant and associated with mutations (Murashige 1974; Bonga 1976; Abbo 78; Anderson 1980).

Willows are of considerable value in horticulture and forestry. This paper describ e micropropagation of *Salix babylonica* Roxb, the weeping willow, through sho oliferation *in vitro*.

Material and methods

plugged ends of the stem explants and the resultant explants (*ca.*
generative bud each were planted on variously modified media. The
sprouted after 7–10 days and developed into a male catkin. After 20–2
senesced and abscissed. However, a vegetative shoot developed from t
position of each reproductive bud (figure 1). Such shoots were utilised

2.2 *Culture medium*

Murashige-Skoog (1962) medium modified to contain half the con
major salts but full minor salts and vitamins and 3% sucrose was u
medium (BM) (Angrish and Nanda 1982). To obtain enhanced shoot
was further supplemented with two cytokinins, *i.e.* kinetin (KN) and
purine (BA), alone and in combination with α-naphthaleneacetic
gibberellic acid-3 (GA) as detailed elsewhere in the text. Media were g
agar and pH was adjusted at 5·8 before autoclaving at 1·1 kg/cm^2 fo

2.3 *Cultural conditions*

Cultures were maintained in an air-conditioned room (27 ± 2°C). Twel
and 12 hr of dark cycles of white light from two 4′ cool-white flu
maintained at a distance of 30 cm from the surface of the culture form
illumination.

3. Results

3.1 *Shoot proliferation in vitro*

Explants (20 replicates per treatment) were cultured on the BM sup
different concentrations (0, 0·5, 1, 2·5, 5 mg/l) of KN and BA in 15·2
tubes, each containing 25 ml of the medium. After 50 days the explant
developing mass of shoots were sub-cultured in 100 ml flasks with 50 m
medium each to meet the increasing requirement of nutrition ar
observations on the number of shoots developing from each explan
after another 50 days, *i.e.* 100 days after the commencement of expe

On BM one or sometimes two shoots developed from the extra-axillar
node of each explant and grew to a considerable extent (figure 1). How


1
2
3

Table 1. Number of shoots developed on the BM with different concentrations of KN and BA after 100 days.

Concentration (mg/l)	KN	BA
0	1·5 ± 0·2	—
0·5	3·1 ± 0·4	9 ± 1
1	5·5 ± 0·6	15·2 ± 2
2·5	4·75 ± 0·5	4·4 ± 0·7
5	3·3 ± 0·5	1·1 ± 0·2

± Standard error.

Table 2. Number of shoots developed on BM-BA medium supplemented with different concentrations of NAA and GA after 100 days.

Concentration (mg/l)	NAA	GA
0 (Control)	14·75 ± 1·8	—
0·5	10·5 ± 0·85	12·35 ± 1·13
1	10·6 ± 1·25	10·3 ± 0·85
2·5	6·35 ± 0·84	5·4 ± 0·57

± Standard error.

regeneration of shoots from the stem explants was eliminated in this ca
23 ± 2 shoots per vessel were produced in 100 days.

3.2 *Procurement of shoots for rooting treatment*

It was found that 2–3 cm long shoots were convenient for rooting trea
the majority of shoots produced on BM-BA medium were 0·5–1 cm
therefore too small for excision and subsequent handling. To obtain sh
length, individual shoot masses produced on BM-BA medium after
transferred to a medium with a low (0·1 mg/l) concentration of BA. T

Table 3. Extension growth of shoots produced on BM-BA medium upon transfer to BM + 0·1 mg/l BA medium after 25 days.

Medium	Shoot length (cm)
BM-BA (Control)	0·83 ± 1·06
BM + 0·1 mg/l BA	3·54 ± 0·37

± Standard error.

ıose developing from the NAA-treated ones which were thick and vigorously growi ubsequent growth of plantlets was also better in the latter case.

4 *Transfer and acclimatization of plantlets in soil*

fine mixture of sand and leaf compost (3:1) filled in earthenware pots (9 cm dia.) w sed as the potting substrate. The pots along with the soil were autoclaved to kill a oil-borne infection. Rooted plantlets were gradually pulled out of the agar mediu nd immersed in water. Agar particles sticking to the root system were removed usin ne brush. The plantlets were allowed to remain in water for about 1 hr to complet ach out sucrose and other organic substances of the medium from their surface. It w bserved that if such a practice was not followed a high proportion of plants is infect ith fungus in the pots.

Plantlets were then planted in pots. Each pot was covered with a polythene bag aintain high humidity around the plants. Pots were suitably watered from time me. Knop's (Knop 1834) nutrient solution (5 ml) was added to each pot at week tervals. After about a week the plants started initiating new leaves. During the 2 eek the polythene bags were removed for 3–4 hr daily to expose the plants onditions of natural humidity. After about 4 weeks when the plants had attainec eight of 7–10 cm, these were transferred to bigger (22 cm dia.) pots and we aintained under natural conditions of day length and temperature. Plants were ke overed by polythene sheet for a major part of the day for another week. Thereaf ey could withstand natural conditions and developed into 20–25 cm long saplin ithin 3 months (figure 5).

concentration of cytokinin is recommended. The practice of transfer
to a rooting (auxin containing) medium generally followed for the
(Anderson 1980) was not necessary in the present case and only shor
auxin is recommended. Papov *et al* (1976) also found that excised sh
rooted upon transfer to a BM after pre-treatment with an auxin. Su
some species appears to be due to their easy-to-root nature. Th
encountered in the establishment of plantlets is the rapid desiccation
the abrupt shift to low humidity conditions in the field. This can be av
covering the plants with polythene. It is our considered opinion that
popular species can be similarly propagated.

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Iorphometric studies in *Datura metel* Linn

A B BHATT*, G V SARATBABU and S C PANDEYA

Department of Biosciences, Saurashtra University, Rajkot 360 005, India
*Department of Botany, University of Garhwal, Srinagar 246 174, India

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Abstract. The various forms/varieties of *Datura metel* have so far been distinguished mai on their morphological characters *viz* flower colour and number of corolla tubes. This pa deals with morphometric analysis of four forms of *D. metel* considering all sorts of possi morphological characters. The results clearly indicate large variations among the four for deserving individual taxonomic identity.

Keywords. *D. metel*; Anderson scatter diagram; taxonomic entities.

Introduction

ıfford (1921) distinguished the varieties of *Datura metel* on the basis of stem a ›wer colour and the number of corolla tubes. Timmerman (1927a, b) examined t ıves, stomatal numbers and seeds of various *Datura* species. Bessis and Guyot (19 tempted to use stomatal characters in systematics and phylogenetic studies ›lanaceae. This paper deals with the application of morphometric method in *D. me* xonomy for supplementing precise variability in statistical terms to the essent orphological variations.

Materials and methods

›ur forms of *D. metel* which differ in flower and stem colour, and in number of coro bes in the flower were collected from various localities of Saurashtra (Gujara ıjasthan, MP and UP. Form 1 is characterised by green stem and simple wh fundibuliform flowers. Form 2 bears violet stem and purple flowers with 2–3 coro

Table 1. Mean and standard deviation of 31 morphological char
(number of observations 20) of *D. metel.*

Characters	Form 1	Form 2	Form 3
Length of largest lateral (cm)	52·3 ± 13·4	76·8 ± 11·5	46·8 ± 14·3
Petiole length of 4th leaf (cm)	3·9 ± 0·9	3·7 ± 0·7	2·6 ± 0·4
Leaf area of 4th leaf (cm^2)	49·5 ± 12·6	52·2 ± 9·8	36·9 ± 10·5
Leaf length/width ratio	1·0 ± 0·1	1·3 ± 0·1	1·4 ± 0·1
Pedicel length (cm)	0·7 ± 0·2	1·3 ± 0·3	0·8 ± 0·1
Calyx length (cm)	5·9 ± 0·3	5·2 ± 0·5	5·6 ± 0·8
Calyx teeth number	3·3 ± 0·5	4·5 ± 0·7	4·1 ± 0·5
Calyx teeth incision (cm)	1·4 ± 0·2	1·3 ± 0·1	1·0 ± 0·2
Corolla length (cm)	16·3 ± 1·3	14·9 ± 1·2	12·7 ± 0·8
Corolla length/Calyx length ratio	2·7 ± 0·3	2·9 ± 0·3	2·3 ± 0·4
Corolla lobes	5·0 ± 0·0	5·4 ± 0·6	5·0 ± 0·0
Acumen number	5·0 ± 0·0	5·5 ± 0·8	5·0 ± 0·0
Acumen length (cm)	0·6 ± 0·0	1·1 ± 0·1	0·7 ± 0·1
Corolla tube length (cm)	15·3 ± 1·1	14·0 ± 1·0	12·0 ± 0·8
Depth of corolla lobing (cm)	1·0 ± 0·4	1·8 ± 0·4	0·7 ± 0·2
Maximum diameter of corolla (cm)	7·8 ± 1·5	6·7 ± 1·5	6·1 ± 0·4
Minimum diameter of corolla (cm)	3·0 ± 0·6	3·8 ± 0·4	3·2 ± 0·3
Stamen number	5·0 ± 0·0	5·2 ± 0·0	5·0 ± 0·0
Stamen length (cm)	13·2 ± 0·8	11·2 ± 0·8	10·8 ± 0·8
Stamenoid number	0·0 ± 0·0	4·2 ± 2·6	0·0 ± 0·0
Anther length	1·2 ± 0·1	1·3 ± 0·1	0·9 ± 0·1
Filament/anther ratio	10·6 ± 1·2	9·0 ± 1·3	11·2 ± 1·3
Adnation of stamen to corolla	7·8 ± 0·6	5·3 ± 0·7	6·6 ± 0·6
Pistil number	1·0 ± 0·0	2·4 ± 1·2	1·0 ± 0·0
Pistil length (cm)	10·1 ± 1·2	11·2 ± 1·0	10·1 ± 0·2
Capsule per plant (average)	6·6 ± 3·1	10·6 ± 4·4	6·1 ± 3·2
Capsule diameter (cm)	3·5 ± 0·2	3·9 ± 1·4	3·2 ± 0·3
Spine length (cm)	0·2 ± 0·0	0·2 ± 0·0	0·2 ± 0·0
Seeds per capsule	281·0 ± 49·0	208·0 ± 12·6	133·1 ± 34·7
Seed width	0·3 ± 0·0	0·3 ± 0·0	0·3 ± 0·0
Seed length	0·4 ± 0·0	0·4 ± 0·0	0·4 ± 0·0

orphological characters was critically intercompared in the following sets:

form 1/form 2	form 2/form 3
form 1/form 3	form 2/form 4
form 1/form 4	form 3/form 4

Computations were done with IBM 360 at the Physical Research Laborator
ımedabad.

Results

1 *Morphological characters*

ıble 1 presents the mean and standard deviation of 31 morphological characters ur forms of *D. metel* and shows that the mean value of each quantitative character ecific for each form.

2 *Anderson scatter diagram*

is evident from figure 1 that all the sets of morphological characters have scatterir points with maximum scattering in four sets (figures 1A, C, D and F). Only in tv mbinations (figures 1B and E) the groups of points fell on a diagonal line indicatir e possibility that although the forms are distinctly separate their genetical stalk cou the same.

3 *Student's t-test*

s shown in table 2, null hypothesis was tested by *t*-test for all morphologic aracters. It is evident that for each character at least one combination has disprov

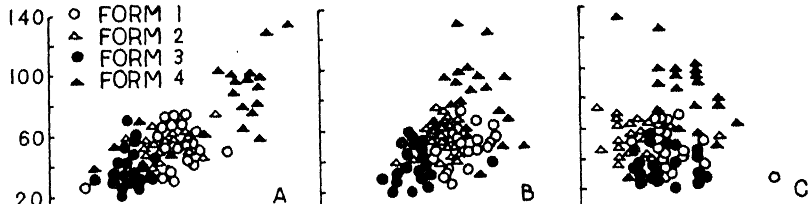

Table 2. *t*-Values of 31 morphological characters in six sets of combina
forms of *D. metel*.

Morphological characters	Forms				
	1:2	2:3	3:4	1:3	2:4
Length of largest lateral	3·094*	4·812*	10·651*	1·248	6·31
Petiole length of 4th leaf	0·903	6·011*	8·054*	6·207*	4·82
Area of 4th leaf	0·739	4·133*	7·152*	3·415*	4·72
Length/Width ratio of 4th leaf	3·539*	2·989	2·465	1·134	4·85
Petiole length	7·121*	1·575	12·061*	1·351	3·71
Calyx length	4·885*	1·022	5·300*	1·705	8·84
Calyx teeth number	6·301*	2·280	7·281*	4·544*	2·93
Calyx teeth incision	2·032	4·500*	8·128*	5·709*	5·49
Corolla length	3·081*	6·307*	10·821*	10·709*	5·19
Corolla length/Calyx length ratio	1·094	4·500*	2·113	4·232*	3·25
Corolla lobes	2·990	2·990	—	—	2·99
Acumen number	2·919	2·919	—	—	2·97
Acumen length	19·175*	15·189*	20·650*	4·580*	6·32
Corolla tube length	3·949*	6·601*	9·072*	10·459*	4·72
Depth of corolla lobing	1·524	1·653	7·562	3·399*	2·64
Maximum diameter of corolla	3·318*	3·131*	14·607*	5·062*	12·21
Minimum diameter of throat	1·123	4·941*	16·405*	2·494	9·95
Stamen number	6·750*	6·750*	—	—	6·75
Stamen length	7·839*	1·130	6·811	9·222*	4·89
Stamenoid number	6·974*	6·974*	—	—	6·97
Anther length	1·217	1·839	16·653*	7·027*	6·96
Filament/anther ratio	4·055*	5·105*	8·818*	1·350	2·19
Adnation of stamen to corolla	2·383	3·101	17·333*	6·451	14·08
Pistil number	5·253*	5·253*	—	—	5·25
Pistil length	2·461	3·816*	0·085	6·299*	3·04
Capsule per plant	2·478	2·875	1·434	0·493	4·47
Capsule diameter	3·837*	5·502*	2·839	3·671	4·72
Spine length	2·373	2·100	3·710*	0·319	5·60
Seed per capsule	3·731*	4·101*	7·651*	11·028*	0·37
Seed width	0·831	0·987	1·414	2·084	0·00
Seed length	0·447	2·847	2·000	3·481*	0·43

giving 351 correlations coefficients. Many of these have become significant a
1–132, form 2–85, form 3–77 and form 4–83.

The results indicate that quite a good number of morphological charact
specific to each form of *D. metel*. Two points emerged from the matrix (i) all t
forms are distinct entities and, (ii) since no environmental correlations co
established with the four forms of *D. metel*, they could be considered for s
taxonomic status.

4. Discussion

The mathematical approach undertaken as above ascertains the differences am
four forms. Anderson scatter diagram clearly marks the morphological variati
shown in figure 1, various sets of characters were considered to prepare the
diagram. If the points are scattered over the plane of the graph, they evidently
to different forms, if the points are assembled over a diagonal line they belong
same population and the variation can be called as environmentally controlled
the groups of the points fall almost on the same line the different populatio
possibly emerged from the same genetical stalk. It is clear in the present study th
the four sets of characters the groups of points fall on a diagonal line indicat
possibility that though these forms exhibit higher degree of diversity i
morphological characters, they might have emerged from the same genetic
sometime in the remote past. Further, it is evident from the results that a large
of correlation coefficients are significant in each form. This implied that som
morphological characters can be considered specific for each form. Similarly *t*-t
applied to see the relationships between the forms with respect to each of
characters. On the basis of the total number of morphological characters
become significant between each of the two forms an inverted tree-like diagr
been prepared which gives a clear picture of the relationship among the fou
(figure 2). Thus it may be concluded that the forms which have emerged
common genetical stalk in the past, have greatly diversified.

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